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(54) Title: **71 HUMAN SECRETED PROTEINS**

(57) Abstract: The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

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## 71 Human Secreted Proteins

### *Field of the Invention*

This invention relates to newly identified polynucleotides, polypeptides  
5 encoded by these polynucleotides, antibodies that bind these polypeptides, uses of  
such polynucleotides, polypeptides, and antibodies, and their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a  
10 membrane, human cells and other eucaryotes are subdivided by membranes into many  
functionally distinct compartments. Each membrane-bounded compartment, or  
organelle, contains different proteins essential for the function of the organelle. The  
cell uses "sorting signals," which are amino acid motifs located within the protein, to  
target proteins to particular cellular organelles.

15 One type of sorting signal, called a signal sequence, a signal peptide, or a  
leader sequence, directs a class of proteins to an organelle called the endoplasmic  
reticulum (ER). The ER separates the membrane-bounded proteins from all other  
types of proteins. Once localized to the ER, both groups of proteins can be further  
directed to another organelle called the Golgi apparatus. Here, the Golgi distributes  
20 the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes,  
and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the  
extracellular space as a secreted protein. For example, vesicles containing secreted  
proteins can fuse with the cell membrane and release their contents into the  
25 extracellular space - a process called exocytosis. Exocytosis can occur constitutively  
or after receipt of a triggering signal. In the latter case, the proteins are stored in  
secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly,  
proteins residing on the cell membrane can also be secreted into the extracellular  
space by proteolytic cleavage of a "linker" holding the protein to the membrane.

30 Despite the great progress made in recent years, only a small number of genes  
encoding human secreted proteins have been identified. These secreted proteins



include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

### *Summary of the Invention*

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

### *Detailed Description*

#### **Definitions**

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA

preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable  
5 of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many  
10 mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment,  
15 polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain  
20 the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the  
25 nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the  
30 polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig

analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard,  
5 Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to  
10 sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon  
15 sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower  
20 percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE,  
25 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress  
30 background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking

reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in

a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

## Polynucleotides and Polypeptides of the Invention

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

5           This gene is expressed primarily in pancreas islet cell tumors.

          In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention.

          Specifically, polypeptides of the invention comprise the following amino acid  
10   sequence:  
PFCSGFFPSLWIYLPFIENVSDLWMGSLSGCALPFCLXVFFLTVSPSAVGLLXF  
AGGPLQTLFAWVSPVEAAEQRLLPVLSSGSFVSEGTCQMPARALLYEVSVG  
PYWEIPPSQDTRRSPTYLRRQSDP (SEQ ID NO: 195). Polynucleotides encoding  
these polypeptides are also encompassed by the invention. Moreover, fragments and  
15   variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
complement thereof are encompassed by the invention. Antibodies that bind  
20   polypeptides of the invention are also encompassed by the invention. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

          Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
25   not limited to, disorders of the pancreas, including cancer and diabetes. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s). For  
a number of disorders of the above tissues or cells, particularly of the pancreas,  
expression of this gene at significantly higher or lower levels may be routinely  
30   detected in certain tissues or cell types (e.g., endocrine, cancerous, or wounded  
tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal  
fluid) or another tissue or cell sample taken from an individual having such a

disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in tumors of pancreatic islet cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, treatment and intervention of such tumors, in addition to other endocrine or gastrointestinal tumors where expression has been indicated. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1099 of SEQ ID NO:11, b is an integer of 15 to 1113, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 2**

This gene is expressed equally in hemangiopericytoma cells, breast lymph node tissue, and bone marrow.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

HEGSCRAPGFSAHKGRGCPSRMTLPSRALASLGVGWGMRLNQVTVSCG  
GSRWSSRVALGAFSWVCGVALVLQPSGGGLGLTSPSEGCWEGELALAVLRA  
PGGSPS (SEQ ID NO: 196). Polynucleotides encoding these polypeptides are also

encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
5 conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Therefore, polynucleotides and polypeptides of the invention are useful as  
10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic disorders, particularly leukemia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For  
15 a number of disorders of the above tissues or cells, particularly of the vascular and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hemolymphoid, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
20 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 104 as residues: Gly-29 to Ser-35, Ser-  
25 63 to Cys-68. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in hemangiopericytoma, breast lymph node, and bone marrow indicates that polynucleotides and polypeptides corresponding to this gene  
30 are useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. Representative uses



are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 969 of SEQ ID NO:12, b is an integer of 15 to 983, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

## **25 FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

The protein product of this gene shares homology with Drosophila, mouse, and human Slit proteins (See Genbank Accession Numbers (protein) AAD25539 and AAD38940; all references available through this accession are hereby incorporated herein by reference; for example Rothberg et al., Genes Dev. 4:2169-87 (1990), Brose K., et al., Cell 1999 Mar 19;96(6):795-806, and Liang Y., et al., J. Biol. Chem. 1999 Jun 18;274(25):17885-92). Slit gene products are secreted proteins that contain both EGF domain and Leucine Rich Repeat domains; and function as ligands for cell-

surface receptors. In *Drosophila*, Slits are important in the development of midline glia and commissural axon pathways. In vertebrates, Slit family proteins have been shown to function as ligands of receptors in nervous system tissue. Slit proteins are thought to be critical for certain stages of central nervous system histogenesis and to have evolutionarily conserved roles in axon guidance (Brose K., et al., *Cell* 1999 Mar 19;96(6):795-806 and Liang Y., et al., *J. Biol. Chem.* 1999 Jun 18;274(25):17885-92). Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with Slit proteins. Such activities are known in the art, some of which are described elsewhere herein.

10        This gene is expressed primarily in human hippocampus.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological, and developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neurological system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neurological, cancerous, or wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25        The tissue distribution within human hippocampus combined with the homology to the *Drosophila* slit protein, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating

diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 959 of SEQ ID NO:13, b is an integer of 15 to 973, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 4

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by

the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

IPLTLPGIFLLIRLFWRLGQSICGPGKLVLPWFCCGCAVISGHCVPRGMPSSW  
LPGCFVLLCLVAVGCQLREWGVGGVSAVGLLALPHLQVLGMRGRGLISGG

5 (SEQ ID NO: 197). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
10 conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
15 16. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 16.

This gene is expressed in KMH2 cells, osteoblasts, fetal spleen, Jurkat membrane bound polysomes, breast, and cerebellum.

Therefore, polynucleotides and polypeptides of the invention are useful as  
20 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer, immune, and skeletal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
25 disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, skeletal, cancerous, or wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the  
30 standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in KMH2 cells, osteoblasts, and fetal spleen indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Expression of this gene product in fetal spleen and T-cells indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1444 of SEQ ID NO:14, b is an integer of 15 to 1458, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 5

The translation product of this gene shares sequence homology with phospholipase A2 which cleaves fatty acids from carbon 2 of glycerol (ref. Prosite pattern documentation for PS2\_HIS). Many snake venoms contain phospholipase A2, which prevents transmission of nerve impulses to muscles by blocking the release of  
15 acetylcholine from the neuron. Therefore, included in this invention as preferred domains are Phospholipase A2 histidine active site domains, which were identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). Phospholipase A2 is an enzyme which releases fatty acids from the second carbon group of glycerol. Structurally, PA2's are small and rigid proteins of 120 amino-acid residues that have  
20 four to seven disulfide bonds. PA2 binds a calcium ion which is required for activity. The side chains of two conserved residues, a histidine and an aspartic acid, participate in a 'catalytic network'. Two different signature patterns for PA2's were developed. The first is centered on the active site histidine and contains three cysteines involved in disulfide bonds. The consensus pattern is as follows: C-C-x(2)-H-x(2)-C [H is the  
25 active site residue].

Preferred polypeptides of the invention comprise a Phospholipase A2 histidine active site domain selected from the following amino acid sequences: CCNQHDCRC (SEQ ID NO: 199), SLTKCCNQHDCRYET (SEQ ID NO: 200), and/or  
LTKCCNQHDCRYETCG (SEQ ID NO: 201). Polynucleotides encoding these  
30 polypeptides are also encompassed by the invention. Further preferred are polypeptides comprising the Phospholipase A2 histidine active site domain of the sequence listed in Table 1 for this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75

- additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues may be N-terminal or C-terminal to the Phospholipase A2 histidine active site domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the
- 5 Phospholipase A2 histidine active site domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to Phospholipase A2 proteins. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with Phospholipase A2 proteins.
- 10 Such activities are known in the art, some of which are described elsewhere herein, or see, for example, McIntosh, et al. J. Biol. Chem. 270 (8), 3518-3526 (1995), incorporated herein by reference.

- In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by
- 15 the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:
- GPAGKEAWIWSWLLPSPGPAPLPSASWGLCGDAPRAAARGPVEPGAARMAL  
LSRPALTLLLLMAAVVRCQEQAQTTDWRATLKTIRNGVHKIDTYLNAALDL  
LGGEDGLCQYKCSDBGSKPFPYGYKPSPPNGCGSPLFGXHLNIGIPSLTKCCN  
20 QHDCRYETCGKSKNDCDEEFQYCLSKICRDVQKTLGLTQHVQACETTVELLF  
DSVIHLGCKPYLDSQRAACRCHYEKTDL (SEQ ID NO: 198). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or
- 25 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the
- 30 invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 4. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 4.

This gene is expressed in a diverse variety of cell types.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders, or metabolism disorders, specifically phospholipase A2 deficiencies. Similarly, polypeptides and antibodies directed to  
10 these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neuromuscular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., pancreas, cancerous and wounded tissues) or bodily fluids (e.g., bile,  
15 lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the  
20 immunogenic epitopes shown in SEQ ID NO: 107 as residues: Gln-23 to Asp-30, Lys-66 to Cys-87. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The ubiquitous tissue distribution and homology to phospholipase A2  
25 indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of neuromuscular disorders. Alternatively, considering the activity of phospholipase A2 as a block for neuro- transmission may suggest that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioural disorders  
30 such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors,



including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Alternatively, the homology to Phospholipase A2 proteins may indicate a potential use for the protein product of this gene in diagnosis, treatment and/or prevention of metabolism disorders, specifically deficiencies in Phospholipase A2. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1991 of SEQ ID NO:15, b is an integer of 15 to 2005, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 6

In a specific embodiment polypeptides of the invention comprise the following amino acid sequence:

GTSSARPRGALPGGSAPSAPHGQLPGRAQPAPVSGPPPTSGLCHFDPAAPWPL  
WPGPWQLPPHPQDWPAPHDPQDWVSFLRSFGQLTLCPRNGTVTGKWRGSH  
VVGLLTTLNFGDGPDRNKTRTFQATVLGSQMGLKGSSAGQLVLITARVTTER  
TAGTCLYFSAVPGILPSSQPPISCSEEGAGNATLSPRMGEECVSVWSHEGLVLT  
KLLTSEELALCGSRLLVLGSFLLFCGLLCCVTAMCFHPRRESHWSRTRL  
(SEQ ID NO: 202). Polynucleotides encoding these polypeptides are also

encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
5 conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, polypeptides comprising the amino acid sequence of  
10 the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:  
ARAPPGPEGLSPEAQPLLPMGNCQAGHNLHLCLAHHPPLVCATLILLGLS  
GLGLGSFLLTHRTGLRT LTSPRTGSLF (SEQ ID NO: 203). Polynucleotides  
15 encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these  
20 polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in a wide variety of tissue types including testes,  
25 cerebellum, dendritic cells, breast cancer, umbilical vein endothelial cells, epididymus, corpus colosum, chronic synovitis, liver hepatome, normal breast, osteoblasts, melanocytes, B cell lymphomas, and to a lesser extent in other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
30 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer, particularly of endothelial tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endothelial, cancerous, or wounded  
5 tissues) or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the  
10 immunogenic epitopes shown in SEQ ID NO: 108 as residues: Thr-52 to Gly-57. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that the protein product of this gene may play a role in the  
15 regulation of cellular division and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the  
20 proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also relies on decisions involving cell differentiation and/or apoptosis in  
25 ~~system formation. Dysregulation of apoptosis can result in inappropriate expansion~~

detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in  
5 modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their  
10 interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
15 related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
20 formula of a-b, where a is any integer between 1 to 929 of SEQ ID NO:16, b is an integer of 15 to 943, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 7

25 In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:  
RFLSVXPQXEVFPFLHPCVCFXGGHPSLLPDPCRAVGGGWEAPRCCLHEALC  
30 QSLGCKABEIVSVSESSSAQRCWYLLRGRKAGGRGPASPVLFAIMRLESLSCH  
LCLACLFRLPATRTVYCMNEAIVDVALGILIESRKQXKACEQPALAGADNP  
EHSPPCSVSPHTSSGSSSEEDSGKQALXPGLSPSQRPGGSSSACSRSPEEEEEE

DVLKYVREIFFS (SEQ ID NO: 204). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. Polynucleotides of the invention do not consist of the nucleic acid sequences shown as GeneSeq Accession Nos: V59595 and V59744, which are hereby incorporated herein by reference.

This gene is expressed primarily in a variety of immune cell types, including stromal cells, dendritic cells, leukocytes, activated T-cells, macrophages, monocytes, neutrophils and to a lesser extent in a variety of other adult and fetal tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous, or wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Expression of this gene product in fetal

tissue and various hematopoietic cancers indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

formula of a-b, where a is any integer between 1 to 1489 of SEQ ID NO:17, b is an integer of 15 to 1503, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 8

When tested against Jurkat T-cell lines, supernatants removed from cells containing this gene activated the NF-kB (Nuclear Factor kB) pathway. Thus, it is likely that this gene activates T-cells through the NF-kB signal transduction pathway. NF-kB is a transcription factor activated by a wide variety of agents, leading to cell  
 10 activation, differentiation, or apoptosis. Reporter constructs utilizing the NF-kB promoter element are used to screen supernatants for such activity. In a specific embodiment polypeptides of the invention comprise the following amino acid sequence:

15 VPGWPRACSPCQADSPRAHPPKLRGILRWAPVPLXCAALCPPLDSGMSMAA  
 CPEAPEPSFLREVPSSPASTQWHRPCNFRQVEANPRKEPKNLVWRDVS LGQX  
 SRTPRGSGLELVRVCGGGMQRDKTVVEERVGEERERERERESLGGAGKHGE  
 MRCVYVRESVGAPGRAGGGGNGVNSVGCVRTVHSGSXPPPSAGVS (SEQ ID  
 NO: 205). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for  
 20 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by  
 25 the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in parts of the brain such as cerebellum and frontal lobe.

Therefore, polynucleotides and polypeptides of the invention are useful as  
 30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disorders. Similarly, polypeptides and antibodies

directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain  
5 tissues or cell types (e.g., neural, cancerous, or wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10       The tissue distribution in cerebellum and frontal lobe indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, prevention and/or treatment of neurodegenerative disease states and behavioural disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example  
15 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms,  
20 hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product  
25 is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies  
30 directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.



Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1498 of SEQ ID NO:18, b is an integer of 15 to 1512, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 9

In a specific embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

TRPGKELNLVFGLQLSMARIGSTVNMNLMGWLYSKIEALLGSAGHTTLGITL  
MIGGITCILSLICALALAYLDQRAERILHKEQGKTGEVIKLTVDKDFSLPLWLIF  
IICVCYYVAVFPFIGLGKVFFTEKFGFSSQAASAINSVVYVISAPMSPVFGLLV  
DKTGKNIWVLC (SEQ ID NO: 206). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in fetal tissue, and to a lesser extent in a variety of adult human tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, fetal abnormalities, particularly developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developing, or cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 111 as residues: Lys-30 to Thr-35. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in fetal tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division. Because of potential roles in proliferation and

differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1641 of SEQ ID NO:19, b is an integer of 15 to 1655, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 10**

The translation product of this gene shares sequence homology with human histiocyte-secreted factor (HSF) which is a novel cytokine that shows in vivo antitumour activity without the cytotoxicity associated with tumour necrosis factor. Furthermore, the translation product of this gene also shares sequence homology with the human endogenous virus S71 gag polyprotein, the sequence of which is believed to represent a transformation locus for several cancers (See Genbank Accession No. pir|A46312|A46312; all references available through this accession are hereby incorporated by reference herein). Similarly, the translation product of this gene also shares homology with B219, a sequence that is expressed in at least four isoforms in very primitive hematopoietic cell populations which may represent a novel hemopoietin receptor (See, e.g., Cioffi, et al. Nat. Med. 2:585-589 (1996), which is hereby incorporated by reference herein). In a preferred embodiment polypeptides of the invention comprise the following amino acid sequence:  
CKDLCSRVLTLSPLLSYDPATSHSPRNTQ (SEQ ID NO: 207). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in tonsil, and colon, and to a lesser extent in a wide variety of human tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune, hematopoietic, and gastrointestinal disorders, particularly tumors of the colon and tonsil. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above

tissues or cells, particularly of the hematopoietic, digestive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, gastrointestinal, or cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 112 as residues: Met-1 to Cys-6. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in tonsil and colon, combined with the homology to human histiocyte growth factor, the human endogenous viral protein, and B219 strongly indicate that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, treatment and/or prevention, of a variety of hematopoietic and immune system disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein.

Expression of this gene product in tonsils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and

graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2511 of SEQ ID NO:20, b is an integer of 15 to 2525, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

ICECWEEECQSCRLKITQPREICRMDFLVLFYLASVLMGLVLICVCSKTHS  
LKGLARGGAQIFSCIPECLQRAXHGLLHYLFHTRNHTFIVLHLVLQGMVYTE  
YTWEVFGYCQELELSLHYLLLPLYLLGVNLFFFTLTCGTNPGIITKANELLFLH  
VYEFDEVMFPKNVRCSTCDLRKPARSKHCSVCNWCVHRFDHHCVVVNNCI  
5 GAWNIRYFLIYVLTLTASAATVAIVSTTFLVHLVVMSDLYQETYIDDLGHLHV  
MDTVFLIQYLFLTFRIVFMLGFVVVLSFLLGGYLLFVLYLAATNQTTNEWYR  
GDWAWCQRCPLVAWPPSAEPQVHRNIHSHGLRSNLQEIFLPAFPCHERKKQE  
(SEQ ID NO: 208). Polynucleotides encoding these polypeptides are also  
encompassed by the invention. Moreover, fragments and variants of these  
10 polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
conditions, to the polynucleotide encoding these polypeptides, or the complement  
there of are encompassed by the invention. Antibodies that bind polypeptides of the  
15 invention are also encompassed by the invention. Polynucleotides encoding these  
polypeptides are also encompassed by the invention.

This gene is expressed primarily in colon and brain and to some extent in all  
tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
20 reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, neurological and digestive disorders. Similarly, polypeptides and  
antibodies directed to these polypeptides are useful in providing immunological  
probes for differential identification of the tissue(s) or cell type(s). For a number of  
25 disorders of the above tissues or cells, particularly of the central nervous system and  
digestive system, expression of this gene at significantly higher or lower levels may  
be routinely detected in certain tissues or cell types (e.g., neurological,  
gastrointestinal, or cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
30 taken from an individual having such a disorder, relative to the standard gene  
expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
individual not having the disorder.

The tissue distribution in brain indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions.

Representative uses are described in the "Regeneration" and "Hyperproliferative

5 Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction,  
10 aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

15 Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

Alternatively, expression of this gene in colon may indicate a role in the detection, prevention and/or treatment of colon disorders such as colon cancer, Crohn's disease, ulcers, and digestive tract disorders in general. Furthermore, the protein may also be  
20 used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
30 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1382 of SEQ ID NO:21, b is an



integer of 15 to 1396, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 12

5           When tested against Reh cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activation site) pathway. Thus, it is likely that this gene activates B-cells through the Jak-STAT signal transduction pathway. GAS is a promoter element found upstream in many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal  
10           transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STATs pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

          This gene maps to chromosome 7, and therefore, may be used as a marker in  
15           linkage analysis for chromosome 7.

          This gene is expressed primarily in brain, and in the developing embryo.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
20           not limited to, neurological, behavioral, immune, and developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and developmental systems, expression of this gene at significantly higher or  
25           lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developing, immune, or cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily  
30           fluid from an individual not having the disorder.

          Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 114 as residues: Lys-60 to Asn-67.

Polynucleotides encoding said polypeptides are also encompassed by the invention.  
Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions.

5 Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome,

10 meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep

15 patterns, balance, and perception. In addition, the tissue distribution in developing embryo indicates that the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo; sexually-linked disorders, or disorders of the cardiovascular system. Alternatively, the biological activity within B-cells indicates that polynucleotides and polypeptides

20 corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Activation of genes within B-cells indicates a role for this protein in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen

25 presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid

30 arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their

interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1055 of SEQ ID NO:22, b is an integer of 15 to 1069, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 13**

This gene maps to chromosome 6, and therefore, may be used as a marker in linkage analysis for chromosome 6.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

LLSFKIRGLRTEDAGWAQSSSGGLCVRGDAFWMPSSSSGLGSPSRPPSSFLCL  
LLLLLPPAALALLFFLDFFPPRAAVSPFLPDHCSARQPRVWRRETLNRSASGL  
GCWARSTEQGAVGVATGTVLDI SLPASCLSLWPPGPSGGI (SEQ ID NO: 209).

25 Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by

the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In a specific embodiment polypeptides of the invention comprise the following amino acid sequence:

5 QLGLCLTSASLPPASRCGHQAPLGASDLSAHHSAPGFSDSYFTMSCQSSLSRA  
EILQCPLVPSVSPPTHL PQGRANKSSRASLPLLPQTHWCLFPSARGWRRGIQSG  
LPPGGSCTSPRSPPQTLHQHITLVNHNTSYWQSPST (SEQ ID NO: 210),  
HQPPCLLPLAVATRPLWGHLTCLPIILHLVSVTLTSPCLANQAFQGQRSYNAL  
WCPLFLLLPTSPKGEQTNHPEPACPCFPKLTGVFSLQHVVGAEFEFSQVFLLD  
10 PVPVLDHLLKLFTSTSHLLIIPHIGKAPAPDSLL EELSLSLATHCKVAVARFT  
(SEQ ID NO: 211). Polynucleotides encoding these polypeptides are also

encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
15 and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

20 Polynucleotides of the invention do not consist of the nucleic acid sequence shown as GeneSeq Accession No. X04377, which is hereby incorporated herein by reference.

This gene is expressed primarily in brain.

Therefore, polynucleotides and polypeptides of the invention are useful as  
25 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, behavioral and neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
30 disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, or cancerous and wounded

tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 115 as residues: Pro-2 to Gly-7, Ser-10 to Ser-16, Pro-52 to Val-62, Arg-64 to Ser-73. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

10 The tissue distribution in brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly,  
15 the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive  
20 compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission,  
25 learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or  
30 immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1644 of SEQ ID NO:23, b is an integer of 15 to 1658, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

#### 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 14

The translation product of this gene was shown to have homology to the lysosomal mannosidase alpha-B protein (See Genebank Accession No. P34098) which is thought to be important in protein metabolism. One embodiment of this gene comprises polypeptides of the following amino acid sequence:  
15 MAAEGSRFSSQSPGLVDRQGPKCDPSRLVSPWGRHGLRILQIGHHHGRDGQH  
EATHHLLRVLRAPRVGKADEGAVDSDPSTPLQLKHEAAHAEDHAQQVHVVR  
RRVVQGRVTFARRGLVPQHFRPPWVRHIVSGHSESKARSRLFRCRNRSFRR  
AS (SEQ ID NO: 212), and/or RLVSPWGRHGLRILQIGHHHGRDGQH  
EATHHLLRVLRA (SEQ ID NO: 213). Polynucleotides encoding these polypeptides  
20 are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement  
25 there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene maps to chromosome 19, and therefore, may be used as a marker in linkage analysis for chromosome 19.

30 This gene is expressed primarily in brain, placenta, fetal liver, and to a lesser extent in most tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological, reproductive, and hepatic disorders. Similarly,

5 polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, hepatic, or cancerous and

10 wounded tissues) or bodily fluids (e.g., bile, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

15 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 116 as residues: Asn-34 to Lys-42, Leu-60 to Trp-70. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

20 The tissue distribution predominantly in brain indicates a role in the detection/treatment of neurodegenerative disease states and behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. Alternatively, the tissue distribution in liver indicates that polynucleotides

25 and polypeptides corresponding to this gene are useful for the detection and treatment of liver disorders and cancers (e.g., hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies,

30 pre-natal disorders and various wound-healing models and/or tissue trauma.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1063 of SEQ ID NO:24, b is an integer of 15 to 1077, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 15

This gene is expressed primarily in spinal cord, Merkel cells, and adipose tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
15 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the nervous and immune systems, particularly those disorders relating to the CNS involving lipid metabolism disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For  
20 a number of disorders of the above tissues or cells, particularly of the nervous and immune systems and adipose tissue, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, immune, or cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken  
25 from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in spinal cord, Merkel cells and adipose tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the  
30 treatment and/or diagnosis of diseases the nervous systems, such as spinal cord injury, neurodegenerative diseases, muscular dystrophy or obesity. Protein, as well as,



antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1191 of SEQ ID NO:25, b is an integer of 15 to 1205, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 16

The translation product of this gene shares sequence homology with the human uncoupling protein-2 which is thought to be important in energy metabolism, obesity, and the predisposition of hyperinsulinemia (See Genbank Accession No. gi|1857278). Recently, another group published on this gene, designating it brain mitochondrial carrier protein-1 (BCMP1) (J Biol Chem 1998 Dec 18;273(51):34611-5). One embodiment of this gene comprises polypeptides of the following amino acid sequence: PTDVLKIRMQAQ (SEQ ID NO: 214), and/or TYEQLKR (SEQ ID NO: 215). An additional embodiment is the polynucleotides encoding these polypeptides.

This gene maps to the X chromosome, and therefore, may be used as a marker in linkage analysis for the X chromosome.

This gene is expressed primarily in manic depression brain tissue, epileptic frontal cortex, human erythroleukemia cell line, T-helper cells, and to a lesser extent in endothelial and amygdala cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the central nervous system or hematopoietic/immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are

useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system or hematopoietic/immune systems, expression of this gene at significantly higher or lower levels may be routinely  
5 detected in certain tissues or cell types (e.g., neural, hemolymphoid, or cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 118 as residues: Ser-34 to Thr-39, Gln-198 to Leu-205. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

15 The tissue distribution in neural tissues combined with the homology to the human uncoupling protein indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection and/or treatment of neurodegenerative disease states and behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome,  
20 schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked  
25 disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Alternatively, given the homology to uncoupling proteins, the gene and/or its translation product may also be used in the diagnosis, treatment, and/or prevention of thermogenesis disorders such as obesity,  
30 cachexia, and hyperinsulinemia. Uncoupling proteins dissipate the proton gradient created from the oxidation of fuels by the electron transport chain, thus releasing stored energy as heat. Dysfunction of thermogenesis can induce disorders such as

obesity and cachexia. It is thought that obesity may result from decreased thermogenesis in humans. Alternatively, cachexia is a metabolic state in which energy expenditure exceeds food intake, for example in anorexia nervosa. Uncoupling proteins may be useful for the treatment and/or prevention of diseases and/or disorders involved with aberrant metabolic and thermogenic pathways. The following method provides for the determination of respiration uncoupling activity of the polypeptides of the present invention, including fragments and variants of the full length proteins. Briefly, yeast are transfected with an expression vector expressing polypeptide of the present invention as previously described by Bouillaud et al., EMBO J., 13:1990 (1994) (incorporated by reference herein in its entirety). Rates of growth in liquid medium of transformed yeast are measured in the presence of galactose, which induces expression, as described in International Publication No. WO 98/31396 (incorporated by reference herein in its entirety). Instantaneous generation times are compared between the polypeptide of the present invention and appropriate controls. An in vivo decrease of membrane potential associated with uncoupling of respiration is analyzed by flow cytometry of yeast labeled with the potential sensitive probe DiOC6 (3) (3,3'-dihexyloxacarbocyanine iodine, Molecular Probes, Eugene, OR). The ability of a polypeptide of the present invention to influence mitochondrial activity and uncouple respiration is thus determined.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1660 of SEQ ID NO:26, b is an integer of 15 to 1674, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

30

**FEATURES OF PROTEIN ENCODED BY GENE NO: 17**

The translation product of this gene shares sequence homology with 55 kD deglycosylated zona pellucida protein which is known to be important in egg fertilization (See Genebank Accession No.R39356). One embodiment of this gene comprises polypeptides of the following amino acid sequence:

- 5 RPRPSASSLARSASLLPAAHGXGVGGAGGGSSXLSRYQQQLQNEEESGEPEQ  
AAGDAPPPYSSISAESAHXFDYKDESGFPKPPSYNVATTLPYDEAERTKAEA  
TIPLVPGRDEDFVGRDDFDDADQLRIGNDGIF (SEQ ID NO: 216),  
RYQQQLQNEEESGEPEQAAGD (SEQ ID NO: 217), and/or  
PGRDEDFVGRDDFDDADQLRIG (SEQ ID NO: 218). Polynucleotides encoding  
10 these polypeptides are also encompassed by the invention. Moreover, fragments and  
variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
15 complement there of are encompassed by the invention. Antibodies that bind  
polypeptides of the invention are also encompassed by the invention. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments of the invention comprise the following amino acid sequence:

- 20 MLTFFMAFLFNWIGFFLSFCLTTSAAGRYGAISGFGLSLIKWILIVRFSTYFPGY  
FDGQYWLVVFLVLGFLFLRGFINYAKVRKMPET FSNLPRTRVLFIY (SEQ  
ID NO: 219). Polynucleotides encoding these polypeptides are also encompassed by  
the invention. Moreover, fragments and variants of these polypeptides (such as, for  
example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
25 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides, or the complement there of are encompassed by the  
invention. Antibodies that bind polypeptides of the invention are also encompassed by  
the invention. Polynucleotides encoding these polypeptides are also encompassed by  
30 the invention.

Preferred polypeptide variants of the invention comprise the following amino acid sequence:

MKKSLENLNRLQVMLLHLTAAFLQRAQHXYFDYKDESGFPKPPSYNVATTLP  
 YDEAERTKAEATIPLVGRDEDFVGRDDFDDADQLRIGNDGIFMLTFFMAFLF  
 NWIGFFLSFCLTTSAAGRYGAISGFGLSLIKWILIVRFSTYFPGYFDGQYWLW  
 WVFLVLGFLFLRGFINYAKVR KMPETFSNLPRTVLFIY (SEQ ID NO: 220),  
 5 MLLHLTAAFLQRAQFSTYFPGYFDGQYWLWWVFLVLGFLFLRGFINYAKV  
 RKMPETFSNLPRTVLFIY (SEQ ID NO: 221),  
 MLTFFMAFLFNWIGFFLSFCLTTSAAGRYGAISGFGLSLIKWILIVRFSTYFPAF  
 MNSLSRSKRTPAGESRCRTQRNNHLL (SEQ ID NO: 222), and/or  
 MKKSLENLNRLQVMLLHLTAAFLQRAHXILTTRMSLGFSPLTM (SEQ ID  
 10 NO: 223). Polynucleotides encoding these polypeptides are also encompassed by the  
 invention. Moreover, fragments and variants of these polypeptides (such as, for  
 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 15 encoding these polypeptides, or the complement thereof are encompassed by the  
 invention. Antibodies that bind polypeptides of the invention are also encompassed by  
 the invention. Polynucleotides encoding these polypeptides are also encompassed by  
 the invention.

When tested against U937 cell lines, supernatants removed from cells  
 20 containing this gene activated the GAS (gamma activating sequence) promoter  
 element. Thus, it is likely that this gene activates myeloid cells, and to a lesser extent,  
 other immune and hematopoietic cells and JAK-STAT signal transduction pathway.  
 GAS is a promoter element found upstream of many genes which are involved in the  
 Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway  
 25 involved in the differentiation and proliferation of cells. Therefore, activation of the  
 Jak-STAT pathway, reflected by the binding of the GAS element, can be used to  
 indicate proteins involved in the proliferation and differentiation of cells.

This gene is expressed primarily in adult kidney, colon adenocarcinoma, and  
 fetal brain, and to a lesser extent, ubiquitously expression in many tissues.

30 Therefore, polynucleotides and polypeptides of the invention are useful as  
 reagents for differential identification of the tissue(s) or cell type(s) present in a  
 biological sample and for diagnosis of diseases and conditions which include, but are

not limited to, disorders of kidney, colon cancers, and central nervous system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the renal and neural systems, and cancers, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., renal, neural, urogenital, or cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 119 as residues: Cys-15 to Gly-36. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution adult kidney, colon adenocarcinoma, and fetal brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of kidney diseases, colon cancers, and disorders of the central nervous system. Additionally, the homology to the zona pellucida protein indicates that the gene product may be used for male contraceptive development, and infertility diagnosis etc. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1951 of SEQ ID NO:27, b is an

integer of 15 to 1965, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 18

5       The translation product of this gene shares sequence homology with the chicken transferrin receptor in addition to a human prostate-specific protein homolog (See Genebank Accession Nos. *pir*|JH0570|JH0570 and *gi*|2565338 (AF026380), respectively). This gene also shares significant homology with both the murine and the rat hematopoietic lineage switch 2 proteins (See Genbank Accession Nos. 10 *g*3169729 and *g*3851632, respectively), which are induced during an erythroid to myeloid lineage switch.

A preferred polypeptide fragment of the invention comprises the following amino acid sequence: MTVMDPKQMNVA~~AA~~VWAVVS~~Y~~VVADMEEMPLRS (SEQ ID NO: 224). Polynucleotides encoding these polypeptides are also 15 encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement 20 there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal tissues, such as liver/spleen and brain.

Therefore, polynucleotides and polypeptides of the invention are useful as 25 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pre-natal disorders, anomalies, deficiencies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of 30 disorders of the above tissues or cells, particularly of the developing fetus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developing, cancerous and wounded tissues) or bodily fluids

(e.g., amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 120 as residues: Arg-31 to Lys-37, Lys-58 to Glu-65, Asp-157 to Gly-168, Ile-219 to Gly-225, Ala-260 to Ser-268, Thr-276 to Glu-282. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the  
10 invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of pre-natal disorders, anomalies and deficiencies.

The homology to the hematopoietic lineage switch 2 proteins also indicates  
15 that the translation product of this gene is useful for the detection and/or treatment of immune system disorders. In addition, the homology to the transferrin receptor indicates that the translation product of the present invention may have utility in the regulation of iron metabolism as well as the numerous genes under the stringent control of physiologic iron levels. Protein, as well as, antibodies directed against the  
20 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of  
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1849 of SEQ ID NO:28, b is an  
30 integer of 15 to 1863, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.



**FEATURES OF PROTEIN ENCODED BY GENE NO: 19**

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the

5 following amino acid sequence:

PRVRSREPVAGAPGCGTAGPPAMATLWGGLRLGSLLSLSCLALSVLLLAHC  
QTPPSDCLHVVEPMPVRGPDVEAYCLRCECKYEERSSVTIKVTIIIYLSILGLLL  
LYMVYLTLEPILKRRLFGHAQLIQSDDDIGDHQPFANAHDVLARSRSRANV  
LNKVEYAAQQRWKLQVQEQRKSVFDRHVVLS (SEQ ID NO: 225).

10 Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
15 encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane  
20 domain at about amino acid position 72 - 88 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 89 to 167 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

25 A preferred polypeptide variant of the invention comprises the following amino acid sequence:

MATLWGGLRLGSLLSLSCLALSVLLLAHCQTPPRISMSDVNVSALPIKKNS  
GHIYNKNISQKDCDCLHVVEPMPVRGPDVEAYCLRCECKYEERSSVTIKVTIII  
YLSILGLLLLYLMVYLTLEPILKRRLFGHAQLIQSDDDIGDHQPFANAHDVLA

30 RSRSRANVLNKVEYGTAALEASSPRAAKSLSLTGMLSSANWGIEFKVTRKKQ  
ADNWKGTDWVLLGFILPC (SEQ ID NO: 226). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and

variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in infant brain tissue, and to a lesser extent in other cell types and tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental and neurodegenerative diseases of the brain and nervous system, such as depression, schizophrenia, Alzheimer's disease, Parkinson's disease, Huntington's disease, mania, dementia, paranoia, addictive behavior, sleep disorders, epilepsy, transmissible spongiform encephalopathy (TSE), Creutzfeldt-Jakob disease (CJD). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, or cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 121 as residues: Gln-110 to Pro-120, Val-152 to Val-159. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in infant brain tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of developmental, degenerative and behavioral conditions of the brain and nervous system. Representative uses are described in the "Regeneration" and

5 "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of schizophrenia, Alzheimer's disease, Parkinson's disease, Huntington's disease, Tourette Syndrome, transmissible spongiform encephalopathy (TSE), Creutzfeldt-Jakob disease (CJD), mania, depression,

10 dementia, paranoia, addictive behavior, obsessive-compulsive disorder and sleep disorders. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show

15 utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1612 of SEQ ID NO:29, b is an integer of 15 to 1626, where both a and b correspond to the positions of nucleotide

25 residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 20**

The translation product of this gene shares sequence homology with a recently published gene Dysferlin, which is thought to be a skeletal muscle gene that is

30 mutated in Miyoshi myopathy and limb girdle muscular dystrophy (See Genbank Accession No. g3600028, and Nat. Genet. 20 (1), 31-36 (1998); all references available through this accession are hereby incorporated by reference herein.)).

This gene is expressed primarily in fetal liver, fetal heart tissue, and T-cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunodeficiency, tumor necrosis, lymphomas, auto-immunities, cancer, inflammation, anemias (leukemia) and liver disorders, vascular disorders, and cancers (e.g., hepatoblastoma, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, developmental, vascular, or cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, bile, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of immune disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g., AIDS), immuno-suppressive conditions (transplantation) and hematopoietic disorders. In addition this gene product may be applicable in conditions of general microbial infection, inflammation or cancer.

Expression in liver may suggest a role for this gene product in the treatment and detection of liver disorders and cancers (e.g., hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells).

Alternatively, the tissue distribution in fetal heart tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound healing. Protein, as well as,

antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Additionally, the homology to the dysferlin gene indicates that polynucleotides and polypeptides corresponding to this gene are useful for diseases related to degenerative myopathies that are characterized by the weakness and atrophy of muscles without neural degradation; such as Duchenne and Becker's muscular dystrophies. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 591 of SEQ ID NO:30, b is an integer of 15 to 605, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

## 20 FEATURES OF PROTEIN ENCODED BY GENE NO: 21

This gene is expressed primarily in haemopoietic cells and tumor cells, such as pancreatic tumor tissue, and to a lesser extent in bladder cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, haemopoietic disorders, diseases of the renal and pancreatic systems, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the haemopoietic, pancreatic, and renal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., pancreas, renal, cancerous and wounded tissues) or bodily

fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of the renal, pancreatic and haemopoietic systems, and cancers thereof. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

10           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
15           cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 917 of SEQ ID NO:31, b is an integer of 15 to 931, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

20

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 22**

This gene is expressed primarily in liver tissue, cancer cells and fetal lung tissue, and to a lesser extent in dendritic cells.

25           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, metabolic disorders, diseases of developing systems and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell  
30           type(s). For a number of disorders of the above tissues or cells, particularly of the fetus, metabolic systems and cancers, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g.,

developing, metabolic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 124 as residues: His-44 to Gly-49. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of the fetus, metabolic systems and cancers.

The tissue distribution also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various would-healing models and/or tissue trauma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1393 of SEQ ID NO:32, b is an integer of 15 to 1407, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 23**

This gene is expressed primarily in central nervous system tissues and cancers, such as endometrial tumors, and to a lesser extent in other tissues and organs.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the CNS and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
10 disorders of the above tissues or cells, particularly of the central nervous system and cancerous tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such  
15 a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 125 as residues: Tyr-16 to Ser-22, Asp-209 to Leu-215. Polynucleotides encoding said polypeptides are also encompassed by  
20 the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of diseases of the central nervous system, as well as  
25 cancers of tissues where expression of this gene has been observed, such as in endometrial tumors.

The tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioural disorders  
30 such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors,



including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1512 of SEQ ID NO:33, b is an integer of 15 to 1526, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 24

The translation product of this gene shares sequence homology with low-density lipoprotein receptor (See, e.g., Genbank Accession No. >dbj|BAA24580.1; all references available through this accession are hereby incorporated by reference herein).

The translation product of this gene also shares sequence homology with a rat homolog of the human CD94 (See, e.g., Genbank Accession No. gb|AAC10220.1; all references available through this accession are hereby incorporated by reference herein).

This gene is expressed primarily in macrophages, eosinophils, neutrophil and other cells of the haemopoietic and immune system.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the immune and haemopoietic systems and diseases of the endothelial and vascular system. Similarly, polypeptides and antibodies directed to

these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, haemopoietic and vascular system, expression of this gene at significantly higher or lower levels may be routinely  
5 detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 126 as residues: Lys-9 to Ala-17, Met-55 to Leu-61, Tyr-105 to Cys-127, Asp-132 to Lys-141, Ser-165 to Tyr-172, Pro-178 to Met-186, His-222 to Gln-227. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also  
15 encompassed by the invention.

The tissue distribution and homology to LDL receptor and rat CD94 homolog indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of the immune, haemopoietic and vascular systems.

20 Moreover, the tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of hematopoietic disorders. This gene product is primarily expressed in hematopoietic cells and tissues, suggesting that it plays a role in the survival, proliferation, and/or differentiation of hematopoietic lineages. Expression of this gene product in  
25 eosinophils and macrophage also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1723 of SEQ ID NO:34, b is an integer of 15 to 1737, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 25

A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

MAAAGRLPSSWALFSPLLAGLALLGVGPVPARALHNVTAELFGAEAWGTLA  
AFGDLNSDKQTDLFVLRERNDLIVFLADQNAPYFKPKVKVSFKNHSALITSVV  
PGDYDGD SQMDVLLTYLPKNYAKSELGAVIFWGQNQTLD PNNMTILNRTFQ  
DEPLIMDFNGDLIPDIFGITNESNQ PQILLGGNLSWHPALTTTSKMRIPHSAPFI  
DLTEDFTADLFLTTLNATTSTFQFEIWENLDGNFSVSTILEKPQNMMVVGQSA  
FADFDGDGHMDHLLPGCEDKNCQKSTIYLVRSGMKQWVPVLQDFSNGKTL  
WGFVPFVDEQQPTEIPITLHIGDYNMDGYPDALVILKNTSGSNQQAFLLENV  
PCNNASCEEARRMFKVYWELTDLNQIKDAMVATFFDIYEDGILDIVLSKGY  
TKNDFAIHTLKNNF EADAYFVKVIVLSGLCS NDCPRR (SEQ ID NO: 227).

Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

When tested against U937 cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates myeloid cells, and to a lesser extent, other immune and hematopoietic cells and tissue cell types, through the JAK-STAT

signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

This gene is expressed primarily in infant brain and placental tissues, and to a lesser extent in several other tissues including cancers.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, brain disorders and diseases of developing systems and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system and fetal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developing, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 127 as residues: Leu-56 to Thr-62, Gln-80 to Pro-87, Gly-106 to Gln-113, Pro-122 to Lys-127, Gln-138 to Asn-146. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in neural tissues and developing tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of the central nervous system, disorders of developing systems, and cancers. The tissue distribution in infant brain tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful

for the detection/treatment of neurodegenerative disease states and behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered  
5 behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

The tissue distribution indicates that polynucleotides and polypeptides  
10 corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or  
15 survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other  
20 cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
30 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2228 of SEQ ID NO:35, b is an

integer of 15 to 2242, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 26

5 Preferred polypeptides encoded by this gene comprise the following amino acid sequence:

MTKREDGGYTFTATPEDFPKKHKAPVIDIGIANTGKFIMTASSDTTVLIWSLK  
 GQVLSTINTNQMNNTTHAAVSPCGRFVASCGFPTDVKVWEVCFGKKGEFQEV  
 VRAFELKGHSAAVHSFAFSNDSRRMASVSKDGTWKLWDTXVEYKKKQDPY  
 10 LLKTGRFEEAAGAXPCRLALSPNAQVLALASGSSIHLYNTRRGEKEECFERVH  
 GECIANLSFDITGRFLASCGDRAVRLFHNTPGHRAMVEEMQGHLKRASNEST  
 RQRLQQQLTQAQETLKSLGALKK (SEQ ID NO: 228). Polynucleotides encoding  
 these polypeptides are also encompassed by the invention. Moreover, fragments and  
 variants of these polypeptides (such as, for example, fragments as described herein,  
 15 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
 under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
 complement thereof are encompassed by the invention. Antibodies that bind  
 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
 20 encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

When tested against U937 Myeloid cell lines, supernatants removed from cells  
 25 containing this gene activated the GAS assay. Thus, it is likely that this gene activates  
 myeloid cells through the Jak-STAT signal transduction pathway. The gamma  
 activating sequence (GAS) is a promoter element found upstream of many genes  
 which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large,  
 signal transduction pathway involved in the differentiation and proliferation of cells.  
 30 Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS  
 element, can be used to indicate proteins involved in the proliferation and  
 differentiation of cells.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 12 - 28 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

5 In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

VIRHEGSTNMELSQMSXLMGLSVLLGLLALMATAAVXRGWLRAGEERSGRP  
10 ACQKANGFPPDKSSGSKKQKQYQRRKEKPQQHNFTHRLAALKSHSGNIS  
CMDFFSSNGKYLATCADDRTIRIWSTKDFLQREHRSMRANVELDHATLVRFP  
DCRAFIVWLANGDTLRVFKMTKREDGGYTFTATPEDFPKKHKAPVIDIGIAN  
TGKFIMTASSDTTVLIWSLKGQVLSTINTNQMNNTAAVSPCGRFVASCFTP  
DVKVWEVCFGKKGEFQEVVRAFELKGHSAAVHSFAFSNDSRRMASVSKDGT  
15 WKLWDTXVEYKKKQDPYLLKTGRFEEAAGAXPCRLALSPNAQVLALASGSS  
IHLYNTRRGEKEECFERVHGECIANLSFDITGRFLASCGDRAVRLFHNTPGHR  
AMVEEMQGHCLKRASNESTRQLQQQLTQAQETLKLKSLGALKK (SEQ ID NO:

229). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for  
20 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by  
25 the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testes, synovial sarcoma and fetal tissues, and to a lesser extent in several other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the reproductive and developing systems and cancers.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, testicular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, seminal fluid, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in testes tissue, synovial sarcoma, and fetal tissues, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of the reproductive and developing systems, and cancers.

The tissue distribution also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence.

This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

Furthermore, the tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders.

Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of



cellular division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and

5 differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. This protein is useful for the treatment, detection, and/or

10 prevention of William's disease. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the

15 above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2221 of SEQ ID NO:36, b is an integer of 15 to 2235, where both a and b correspond to the positions of nucleotide

25 residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 27**

Preferred polypeptides encoded by this gene comprise amino acids 1-363, 2-363, 4-363, 5-363, 30-363, 31-363, 32-363, 75-363, 76-363 and 82-363 of the

30 following amino acid sequence:

MSVMVVRKKVTRKWEKLPGRNTFCCDGRVMMARQKGIFYLTLFLILGTCTL  
FFAFECRYLAVQLSPAIPVFAAMLFLFSMATLLRTSFSDPGVIPRALPDEAAFI

MEIEATNGAVPQGQRPPPRIKNFQINNQIVKLKCYTCKIFRPPRASHCSICDN  
CVERFDHHCPWVGNCVGKRNRYFYLFILSLSLLTIYVFAFNIVYVALKSLKI  
GFLETLKETPGTVLEVLCFFTLWSVVGLTGFHTFLVALNQTTNEDIKGSWTG  
KNRVQNPYSHGNIVKNCCEVLCGPLPPSVLDRRGILPLEESGSRPPSTQETSSS  
5 LLPQSPAPTELNSNEMPEDSSTPEEMPPEPPEPPQEAAEAEK (SEQ ID NO:

230). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
10 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 A preferred polypeptide-variant of the invention comprises the following amino acid sequence:

MLFLFSMATLLRTSFSDPGVIPRALPDEAAFIEMEIEATNGAVPQGQRPPPRIK  
NFQINNQIVKLKCYTCKIFRPPRASHCSICDNCVERFDHHCPWVGNCVGKR  
NYRYFYLFILSLSLLTIYVFAFNIVYVALKSLKIGFLETLKGNSWNCSPHLLL

20 YTLVRRGTDWISYFPRGSQPDNQ (SEQ ID NO: 231). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
25 under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

30 This gene is expressed primarily in ovarian and endometrial tumors, fetal liver, spleen and brain tissues, and to a lesser extent in several other tissues and organs.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the developing systems, and cancers of the female reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developing, female reproductive and fetal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, developing, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 129 as residues: Pro-44 to Lys-54, Cys-88 to His-95, Val-103 to Tyr-108, Gln-181 to Ser-190, Thr-192 to Ile-206, Glu-233 to Ser-245, Ser-252 to Ala-286. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in developing systems indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of disorders of developing and fetal systems, and cancers. Furthermore, the tissue distribution in ovarian and endometrial tumor tissues indicates that the translation product of this gene is useful for the detection, diagnosis, and/or treatment of cancers of the female reproductive system. Accordingly, preferred are antibodies which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting these tumors. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting these tumors in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody

binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2957 of SEQ ID NO:37, b is an integer of 15 to 2971, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 28**

This gene is expressed primarily in normal and cancerous colon tissue, macrophages, endothelial cells and placental tissue, and to a lesser extent in several other tissues and organs.

20 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, colon cancer and gastrointestinal disorders, immune disorders, vascular diseases and disorders of developing systems. Similarly, polypeptides and antibodies  
25 directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, vascular and developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, gastrointestinal,  
30 developmental, vascular, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 130 as residues: Thr-27 to Ser-33.

5 Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in macrophage, endothelial and placental tissues, and normal and cancerous colon tissues, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of immune, gastrointestinal and vascular disorders and diseases. Expression of this gene product in colon tissue indicates involvement in digestion, processing, and elimination of food, as well as a potential role for this gene as a diagnostic marker or causative agent in the development of colon cancer, and cancer in general. Accordingly, preferred are antibodies which specifically bind a portion of the translation product of this gene.

15 Also provided is a kit for detecting colon cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting colon cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

25 Alternatively, the tissue distribution in placental tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta and endothelial cells also indicates that this gene product may be produced more generally

in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body.

Additionally, expression of this gene product in macrophage also strongly indicates a role for this protein in immune function and immune surveillance. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of  $a-b$ , where  $a$  is any integer between 1 to 1149 of SEQ ID NO:38,  $b$  is an integer of 15 to 1163, where both  $a$  and  $b$  correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where  $b$  is greater than or equal to  $a + 14$ .

**FEATURES OF PROTEIN ENCODED BY GENE NO: 29**

The translation product of this gene shares homology with HNK-sulfotransferase, which directs glycan synthesis (see, e.g., Genbank Accession no. AF033827; all references available through this accession are hereby incorporated by  
5 reference herein).

This gene is expressed primarily in activated T cells, osteoclastoma, and glioblastoma, and to a lesser extent in various other normal and transformed cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as  
10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammation, immune defects, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
15 disorders of the above tissues or cells, particularly of the immune and hemopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such  
20 a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 131 as residues: Pro-32 to Gly-48, Gln-63 to Thr-69, Pro-77 to Trp-84, Val-88 to Leu-94. Polynucleotides encoding said  
25 polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in T-cells and various types of neoplasms indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, study and/or treatment of inflammatory and general immune defects, and  
30 various types of neoplasms. Expression of this gene product in T cells strongly indicates a role for this protein in immune function and immune surveillance. This gene product may be involved in the regulation of cytokine production, antigen

presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, the tissue distribution in various cancerous tissues indicates that the translation product of the gene is useful for the detection, diagnosis, and/or treatment of these cancers, as well as cancers of other tissues where expression has been observed. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1918 of SEQ ID NO:39, b is an integer of 15 to 1932, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 30**

Preferred polypeptides of the invention comprise the following amino acid sequence:

LHECLPGSISYLHPRTPLWLCLPPQHLSFSTFSPPWQPAMSPVPGTGGPPCGL  
(SEQ ID NO: 232), and/or  
MLPLLICLLPAIEGKNCLRCWPELSALIDYDLQILWVTPGPPTELSQSIHSLFLE



DNNFLKPWYLDRDHLEEETAKFFTQVHQAIKTLRDDKTVLLEEIYTHKNLFT  
ERLNKISDGLKEKGAPPLHECLPGSISYLHPRTPLWLCLPPQHLSFSTFSPPWQP  
AMSPVPGTGGPPCGL (SEQ ID NO: 233). Polynucleotides encoding these  
polypeptides are also encompassed by the invention. Moreover, fragments and  
5 variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
complement thereof are encompassed by the invention. Antibodies that bind  
10 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in infant brain, testes and activated T cells,  
and to a lesser extent in various other normal and transformed cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as  
15 reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, neurological, reproductive and inflammatory conditions. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s). For  
20 a number of disorders of the above tissues or cells, particularly of the neural, immune  
and male reproductive systems, expression of this gene at significantly higher or  
lower levels may be routinely detected in certain tissues or cell types (e.g., neural,  
immune, reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
25 taken from an individual having such a disorder, relative to the standard gene  
expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the  
immunogenic epitopes shown in SEQ ID NO: 132 as residues: Gly-41 to Leu-46,  
30 Asp-67 to Thr-75, Ile-114 to Ala-123. Polynucleotides encoding said polypeptides are  
also encompassed by the invention. Antibodies that bind said polypeptides are also  
encompassed by the invention.

The tissue distribution in infant brain tissue, testes tissue, and activated T-cells, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the study, diagnosis, and/or treatment of neurological, reproductive and immune system disorders. Expression of this gene product in T-cells indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell type.

Alternatively, the tissue distribution in testes tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Furthermore, the tissue distribution in infant brain tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and

behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 867 of SEQ ID NO:40, b is an integer of 15 to 881, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 31

The translation product of this gene shares sequence homology with some human and rodent melanoma and leukocyte specific antigens (see, for example, Genbank accession nos: gi|189384, gi|205898 and gi|180926).

In addition, the translation product of this gene shares sequence homology with Tetraspan protein (see, for example, Genbank accession number: GI 3152703). Therefore, it is likely that the polypeptide of this gene shares some biological functions, such as cell-to-cell signaling, adhesion, proliferation, and differentiation with Tetraspan.

The polypeptide of this gene has been determined to have two transmembrane domains at about amino acid position 52-68 and 197 - 213 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed

that the protein product of this gene shares structural features to type IIIa membrane proteins.

The transmembrane 4 superfamily (TM4SF) or tetraspan superfamily has at least 16 members (including CD9, CD20, CD37, CD53, CD63, CD81, CD82, A15, CO-029, Sm23, RDS, Uro B, Uro A, SAS, Rom-1, PETA3, and YKK8), is the second biggest subfamily among CD antigen superfamily. and activation antigen of T- cells. All TM4SF member contains four putative transmembrane domains, two extracellular loops, and two short cytoplasmic tails. They are variously expressed on Immature, early, mature, activated lymphocytes, monocytes, macrophages, granulocytes, platelets, eosinophils, basophils, certain leukemic and lymphoma cells, and a variety of other cells and tissues. CD9 cell surface protein is expressed by both hematopoietic and neural cells, and may play a role for CD9 in intercellular signaling in the immune and nervous system. CD63 is a 53-Kd lysosomal membrane glycoprotein that has been identified as a platelet activation molecule, which play important role in cell adhesion of platelets and endothelial cells. Increased mRNA for CD63 antigen was found in atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits, suggesting a potential role of CD63 in progression of atherosclerosis. CD63 is also a mast cell marker. CD82 was originally identified as the target of several mAbs inhibitory to syncytium formation induced by human T-cell leukemia virus type I (HTLV-I), the etiological agent of adult T-cell leukemia. Therefore, this gene could be a target for the development of a drug for this leukemia. CD81 is the target of an antiproliferative antibody. A diverse group of human cell lines, including hematolymphoid, neuroectodermal, and mesenchymal cells, express the CD81 protein. Many of the lymphoid cell lines, in particular those derived from large cell lymphomas, were susceptible to the antiproliferative effects of the antibody. CD81 may therefore play an important role in the regulation of lymphoma cell growth. CD9, CD20, CD37, CD63, CD81 and CD82 have been implicated in the regulation of cell growth, adhesion, and signal transduction of B, T lymphocytes and some other non-lymphoid cells. They associate with CD2, CD21, CD4, CD8, MHC Class II molecules, integrins, function as co-receptor for T, B and other lymphoid cells.

Some TM4SF are leukocyte antigens, highly expressed in activated leukocytes, lymphocytes, are highly specific surface marker for lymphoblastic

leukemia, lymphoma, melanoma, and neuroblastoma. CD9 has been shown to be involved in cell motility and tumor metastasis. These antigen could be a valuable immunogen or target to implement active and passive immunotherapy in patients with cancer. Others have been shown to be involved in inhibition of prostate cancer metastasis. This gene has close homology to C33 antigen (CD82), which is a member of the transmembrane 4 superfamily (TMSF) and activation antigen of T- cells. C33 Ag (CD82) was originally identified as the target of several mAbs inhibitory to syncytium formation induced by human T-cell leukemia virus type I (HTLV-I), the etiological agent of adult T-cell leukemia. Therefore, this gene could be very important target for developing drug for leukemia. Other members of this family are Sm23, CO-029, R2, TAPA-1, CD9, CD37, CD53, and CD63. CD63 is a 53-Kd lysosomal membrane glycoprotein that has been identified as a platelet activation molecule. There are strong evidence indicating that CD63 and Pltgp40, a platelet membrane glycoprotein are the same molecule and that CD63/Pltgp40 is identical to the well-characterized, stage-specific melanoma-associated antigen ME491. These antigens could be valuable immunogens or target to implement active and passive immunotherapy in patients with cancer.

This gene is expressed primarily in fetal tissue (kidney, heart, liver, spleen, brain), macrophages, dendritic cells, retina and to a lesser extent in various other tissues, mostly of lymphoid origin or epithelial cell types. In addition This gene is expressed in cancerous tissues (e.g. breast).

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders and cancers in a variety of organs and cell types. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, proliferating, immune, hematopoietic, integumentary, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine,

synovial fluid, spinal fluid, or amniotic fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 133 as residues: Tyr-123 to Tyr-131, Cys-134 to Ser-145, Tyr-234 to Tyr-244. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

10 The tissue distribution fetal cells and tissues and homology to tumor antigens indicates that polynucleotides and polypeptides corresponding to this gene are useful for study, treatment and diagnosis of lymphoid and epithelial disorders and neoplasms. Additionally, tissue distribution in immune cells and other tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful  
15 for the diagnosis and treatment of disorders affecting hematopoiesis, including cancers. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell  
20 lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders  
25 including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as  
30 autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the

protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1918 of SEQ ID NO:41, b is an integer of 15 to 1932, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 32

The translation product of this gene shares limited sequence homology with VEGF which is thought to be important in regulation of endothelial cell growth. Therefore, it is likely that the protein encoded by this gene would share some similar biological functions.

When tested against U937 Myeloid cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates myeloid cells, and to a lesser extent, other immune and hematopoietic cells and tissue cell types, through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

This gene is expressed in brain.



Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, nervous system disease and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 134 as residues: Thr-25 to Pro-46. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission,

learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1150 of SEQ ID NO:42, b is an integer of 15 to 1164, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 33

The translation product of this gene shares sequence homology with human p150 which is thought to be important in signal transduction in neuronal cells. Therefore, it is likely that the protein encoded by this polynucleotide would share some similar biological functions with p150.

This gene is expressed primarily in whole embryo and cerebellum.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological and growth defects/disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph,

serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of central nervous system, neurodevelopmental, cognitive, and memory disorders. The tissue distribution also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease  
10       states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis,  
15       encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and  
20       perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to  
25       isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates  
30       this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are

described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.

The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1091 of SEQ ID NO:43, b is an integer of 15 to 1105, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 34**

5 This gene is expressed primarily in PMA stimulated HL-60 cells and to a lesser extent in 6 week embryo.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders affecting cell differentiation, particularly hematopoietic disorders and/or defects. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the metabolic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 136 as residues: Pro-61 to Asp-68. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the study of cellular differentiation and for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia. The tissue distribution also indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation;

survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

5        Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Additionally, the expression within  
10        embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below  
15        and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal  
20        muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders  
25        and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be  
30        used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents

that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1248 of SEQ ID NO:44, b is an integer of 15 to 1262, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 35**

This gene is expressed primarily in colon.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders and/or defects of the digestive tract including but not limited to cancers of the gastrointestinal tract. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the

digestive system particularly disorders involving the colon. Further, expression of this gene product in colon tissue indicates involvement in digestion, processing, and elimination of food, as well as a potential role for this gene as a diagnostic marker or causative agent in the development of colon cancer, and cancer in general. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the colon and/or other gastrointestinal tissue including, but not limited to, stomach, small intestine, large intestine, and rectum.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 503 of SEQ ID NO:45, b is an integer of 15 to 517, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 36

This gene is expressed primarily in blood cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a



disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 138 as residues: Pro-19 to Cys-29, Thr-35 to Glu-44, Val-72 to Lys-78. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis and/or treatment of disorders of the immune and hematopoietic system. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:46 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 844 of SEQ ID NO:46, b is an integer of 15 to 858, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 37

This gene is expressed in multiple tissue systems such as brain, immune cells, prostate, uterus, testes, placenta, and fetal heart as well as in cancerous tissues such as ovarian tumors.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the immune, reproductive, urogenital, and central nervous system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, reproductive, urogenital, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 139 as residues: Tyr-33 to Lys-38. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the immune, urogenital, reproductive, and central nervous systems. The tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of diseases of the central nervous system, as well as cancers of tissues where expression of this gene has been observed, such as in ovarian tumors.

The tissue distribution in central nervous system tissues also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the

gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. The tissue distribution in uterus indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating female infertility. The protein product is likely involved in preparation of the endometrium of implantation and could be administered either topically or orally.

Alternatively, this gene could be transfected in gene-replacement treatments into the cells of the endometrium and the protein products could be produced. Similarly, these treatments could be performed during artificial insemination for the purpose of increasing the likelihood of implantation and development of a healthy embryo. In both cases this gene or its gene product could be administered at later stages of pregnancy to promote healthy development of the endometrium. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. The tissue distribution in testes indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 6093 of SEQ ID NO:47, b is an integer of 15 to 6107, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 38**

This gene is expressed in a wide range of tissue systems such as brain, immune cells, fetal liver, kidney, testes, breast, and pancreas as well as cancerous  
5 tissue such as ovarian tumors.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the central nervous system, immune system, urogenital,  
10 and reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells; particularly of the immune and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain  
15 tissues or cell types (e.g., immune, CNS, urogenital, reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 140 as residues: Met-1 to Ser-7, Asp-32 to Pro-43, Ser-96 to Arg-102. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

25 The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the immune, reproductive, urogenital and central nervous systems.

The tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the  
30 treatment and/or diagnosis of diseases of the central nervous system, as well as cancers of tissues where expression of this gene has been observed, such as in ovarian tumors.

The tissue distribution in central nervous system tissues also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioural disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, 5 panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of 10 immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene 15 product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency 20 diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue 25 injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem 30 cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate

ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 689 of SEQ ID NO:48, b is an integer of 15 to 703, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 39**

This gene is expressed primarily in macrophages and fetal cells and to a lesser extent in cancerous ovarian tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune diseases, disorders of developing tissues, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of developmental abnormalities and disorders of the immune systems.



The tissue distribution cancerous ovaries indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and intervention of these tumors.

Expression of this gene product in macrophage cells strongly indicates a role for this protein in immune function and immune surveillance. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). This gene product may have clinical utility in the treatment of immune dysfunction; in the correction of autoimmunity; in immune modulation; and in the control of inflammation.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem

cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

The tissue distribution also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, diagnosis, and/or prevention of various skin disorders such as melanomas. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of  $a-b$ , where  $a$  is any integer between 1 to 625 of SEQ ID NO:49,  $b$  is an integer of 15 to 639, where both  $a$  and  $b$  correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where  $b$  is greater than or equal to  $a + 14$ .

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 40

This gene is expressed primarily in neutrophils, bone marrow, brain, and fetal cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic disorders, Limbic system dysfunction/defects and disorders of the immune system and developing systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, Limbic system and developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 142 as residues: Ala-84 to Gln-93. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the immune, Limbic system, CNS and developing systems. Expression of this gene product in bone marrow, eosinophils, and neutrophils strongly indicates a role for this protein in hematopoiesis and immune surveillance. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). This gene product may have clinical utility in the treatment of immune dysfunction; in the correction of autoimmunity; in immune modulation; and in the control of inflammation.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem

cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 853 of SEQ ID NO:50, b is an integer of 15 to 867, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 41

When tested against MVEC endothelial cell lines, supernatants removed from cells containing this gene activated the expression of ICAM-1. Thus, this gene activates signal transduction pathways which upregulate cell-surface expression and/or secretion of ICAM-1. ICAM-1 - is found on the cell surface of endothelial cells, smooth muscle cells, epithelial cells, and fibroblasts. It binds to its ligand, LFA-1, a heterodimer complex that is a member of the leukocyte integrin family of cell adhesion receptors. Inflammatory mediators and cytokines, such as, IL-1, TNF $\alpha$  and IFN $\gamma$ , stimulate ICAM-1 expression on vascular cells, in addition to the aforementioned cells and tissue cell types. Polypeptides which increase ICAM expression are useful in the treatment of cancer, cardiovascular, autoimmune and inflammatory diseases and/or disorders.

This gene is expressed primarily in ovary and to a lesser extent in fetal tissue, colon, and immune cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, ovarian cancer, gastrointestinal and immune system disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, gastrointestinal, immune, cancerous and wounded tissues) or bodily fluids (e.g.,

lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 143 as residues: Ile-23 to Ala-29. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides  
10 corresponding to this gene are useful for diagnosis and treatment of ovarian cancer and related metastases. The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are also useful for treating female infertility. Additionally, the tissue distribution in colon tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment  
15 of disorders involving the gastrointestinal tract. This may include diseases associated with digestion and food absorption, as well as hematopoietic disorders involving the Peyer's patches of the small intestine, or other hematopoietic cells and tissues within the body. Similarly, expression of this gene product in colon tissue indicates again involvement in digestion, processing, and elimination of food, as well as a potential  
20 role for this gene as a diagnostic marker or causative agent in the development of colon cancer, and cancer in general.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention  
25 of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell  
30 death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of

potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1555 of SEQ ID NO:51, b is an integer of 15 to 1569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 42

The translation product of this gene shares sequence homology with retrovirus-related reverse transcriptase pseudogene. In addition, this gene shares homology with human interferon-beta (Genseq accession number T35524; all references available through this accession are hereby incorporated herein by



reference), therefore, it is likely that this gene and the protein encoded by this gene shares some similar biological functions with this protein.

This gene is expressed primarily in frontal cortex.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
10 disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative  
15 to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in frontal cortex and homology to retrovirus-related reverse transcriptase pseudogene and human interferon-beta indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis  
20 and treatment of neurodegenerative diseases of the brain, particularly of the frontal cortex.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions.  
25 Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, cystic fibrosis, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating  
30 diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder,

learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse  
5 formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may  
10 show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of  
15 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1182 of SEQ ID NO:52, b is an  
20 integer of 15 to 1196, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 43**

This gene is expressed primarily in immune cells, brain, fetal tissue, and  
25 cancerous tissues (such as testes, stomach, lung, pancreas, ovaries) and to a lesser extent in other numerous tissues including, but not limited to, testes and kidney.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
30 not limited to, neurodegenerative diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of

the above tissues or cells, particularly of the central nervous system and immune cells expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or  
5 another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 145 as residues: Lys-23 to Lys-35,  
10 Met-46 to Tyr-52. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of  
15 neurodegenerative disorders of the frontal cortex, as well as, cancer or a number of tissues including but not limited to testes, stomach, lung, pancreas, and ovaries.

The tissue distribution also indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions.  
20 Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia,  
25 trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene  
30 product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell

death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 931 of SEQ ID NO:53, b is an integer of 15 to 945, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 44

This gene is expressed primarily in epithelioid sarcoma and to a lesser extent in pancreatic carcinoma, aorta endothelial cells induced with TNF-alpha, and amniotic cells induced with TNF. This gene is also expressed, to a lesser extent, in cancerous lung and ovary tissue and fetal tissue.

5           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, epithelioid sarcoma and related cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological  
10 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., amniotic, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another  
15 tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 146 as residues: Tyr-39 to Arg-51.  
20 Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of certain cancers, including epithelioid sarcoma and pancreatic carcinoma.

25           The tissue distribution in tumors of lung, ovary, and pancreas origins indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated.

Moreover, the expression within embryonic tissue and other cellular sources  
30 marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative

conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis,

hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 474 of SEQ ID NO:54, b is an integer of 15 to 488, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 45**

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

PPVPPWISLPLTGSPPRPGFVPVSPFCFSPMTNGHQVLLLLLLTSAVAAGPWPQ



VHAGQWGWMLPPGLPSVQARSGLGGLPGGPQWVPGGARGY (SEQ ID NO: 234). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 5 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by 10 the invention.

This gene is expressed primarily in fetal and infant tissue, particularly infant brain and fetal liver/spleen libraries, and to a lesser extent in breast, ovary tumor, pharynx carcinoma, endometrial stromal cells, thymus, islet cell tumors, and adult cerebellum.

15 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological 20 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and breast, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, developmental, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, amniotic fluid, 25 synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in developing cells and tissues indicates that 30 polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders. The expression within cellular sources marked by proliferating cells indicates this protein may play a role in

the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the “Hyperproliferative Disorders” and “Regeneration” sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

formula of a-b, where a is any integer between 1 to 2846 of SEQ ID NO:55, b is an integer of 15 to 2860, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 46

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

10 IQQWGDSVLGRRCDLLQLYLQRPELRVPVPEVLLHSEGAASSSVCKLDGLI  
 HRFITLLADTSDSRALENRGADASMACRKLAVAHPLLLLRHLPMAALLHGR  
 THLNFQEFRQQNHLSCFLHVLGLLELLQPHVFRSEHQGALWDCLLSFIRLLLN  
 YRKSSRHLAAFINKFVQFIHKYTTYNAPAAISFLQKHADPLHDLSDNSDLVM  
 LKSLLAGLSLPSRDDRTDRGLDEEGEEESSAGSLPLVSVSLFTPLTAAEMAPY  
 15 MKRLSRGQTVEDLLEVLSDIDEMSRRRPEILSFFSTNLQRLMSSAECCRNLA  
 FSLALRSMQNPSIAAAFLPTFMYCLGSQDFEVVQTALRNLPEYALLCQEHA  
 AVLLHRAFLVGMYGQMDPSAQISEALRILHMEAVM (SEQ ID NO: 235).

Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example,  
 20 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by  
 25 the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in breast cancer, and to a lesser extent in a variety of other cancers, including uterine cancer, synovial sarcoma, and pharynx carcinoma.

30 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are

not limited to, breast cancer; proliferative diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the breast, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, breast, proliferative, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, breast milk, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 148 as residues: Glu-35 to His-41, Ser-62 to Ala-67, Pro-145 to Leu-155, Glu-157 to Ser-163, Arg-190 to Val-197, Asp-208 to Pro-215, Ser-247 to Pro-252. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in breast cancer tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of cancer. Elevated expression of this gene product in cancers, such as breast cancer, suggest that it may be involved in the abnormal proliferation of cells, dedifferentiation, angiogenesis, and other processes that accompany the development of cancer. Thus, therapeutics targeted against this gene product may be useful therapeutic products in and of themselves.

Alternately, expression of this gene product at elevated levels in breast tissue may be reflective of expression within breast lymph nodes, and may suggest a hematopoietic role for this protein. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired

immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.

10 The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

15 Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:56 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1545 of SEQ ID NO:56, b is an integer of 15 to 1559, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 47**

30 The translation product of this gene shares limited sequence homology with cytochrome-c oxidase. An alternative embodiment is the polypeptide comprising the following amino acid sequence:

MLLKHLQRMVSVQVKASALKVVTLTANDKTSVSFSSLPGQGVYINVIVWD  
PFLNTSAAAYIPAHTYACSFEEAGEGSCASLGRVSSKVFFTLFALLGFFICFFGHR  
FWKTELEFFIGFIIMGFFFYILITRLTPIKYDVNLILTAVTGSVGGMFLVAVVWR  
FGILSICMLCVGLVLGFLISSVTFFTPLGNLKFHDDGVFWVTFSCIALIPVVF  
5 MGCLRILNLTTCGVIGSYSVVLADSYWSTSLSYITLNVLKRALNKDFHRAFTN  
VPFQTNDFIILAVWGMLAVSGITLQIRREGRPFPPHPYKLWKQERERRVTNI  
LDPSYHIPPLRERLYGRLTQIKGLFQKEQPAGERTPLLL (SEQ ID NO: 236).

Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example,  
10 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides, or the complement thereof are encompassed by the  
invention. Antibodies that bind polypeptides of the invention are also encompassed by  
15 the invention. Polynucleotides encoding these polypeptides are also encompassed by  
the invention.

In another embodiment, polypeptides comprising the amino acid sequence of  
the open reading frame upstream of the predicted signal peptide are contemplated by  
the present invention. Specifically, polypeptides of the invention comprise the  
20 following amino acid sequence:  
WARLRGPGAHARTSPQPWRGPSPAQAAMGFLQLLVVXVLXSEHRVAGAAE  
VFGNSSEGLIEFSVGKFRYFELNRPFEEAILHDISSNVTLIFQIHSQYQNTTVS  
FSPRRRSPTM (SEQ ID NO: 237). Polynucleotides encoding these polypeptides are  
also encompassed by the invention. Moreover, fragments and variants of these  
25 polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
conditions, to the polynucleotide encoding these polypeptides, or the complement  
thereof are encompassed by the invention. Antibodies that bind polypeptides of the  
30 invention are also encompassed by the invention. Polynucleotides encoding these  
polypeptides are also encompassed by the invention.

This gene is expressed primarily in keratinocytes, brain, and spinal cord and to a lesser extent in hematopoietic cells and tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disorders; hematopoietic disorders; integumentary disorders; immune dysfunction; learning disabilities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., integumentary, neural, developmental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain and spinal cord cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of neurological and hematopoietic disorders. For example, elevated levels of expression of this gene product in brain and spinal cord indicates that it may be involved in neurodegenerative disorders. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the

brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Alternately, expression of this gene product in hematopoietic cells indicates that it may be involved in the proliferation, differentiation, survival, and activation of all hematopoietic lineages, including stem and progenitor cells.

Expression of this gene product in keratinocytes indicates that it may be involved in normal skin function, and could be involved in skin disorders, dermatitis, and fibrosis. The protein is useful in detecting, treating, and/or preventing congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e., wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis; erysipelas, impetigo, tinea, athlete's foot, and ringworm).

Moreover, the protein product of this gene may also be useful for the treatment or diagnosis of various connective tissue disorders (i.e., arthritis, trauma, tendonitis, chondromalacia and inflammation, etc.), autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, and chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.



Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:57 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2050 of SEQ ID NO:57, b is an integer of 15 to 2064, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 48**

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:  
 PRVRPASPPVRSPARWGSMAGSPLLWGPRAGGVGLLVLLLLGLFRPPPALCA  
 RPVKEPRGLSAASPPLARLALLAASGGQCPEVRRRGRRCRPGAGAGASAGAER  
 QERARAEAQRLRISRRASWRSCCASGAPPATLRLWAWTTTPTRLQRSSLALC  
 SAPALTLP (SEQ ID NO: 238). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human pituitary and to a lesser extent in pineal gland, and other areas of the brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pituitary dysfunction; abnormal growth; neurological defects; insufficient milk secretion; abnormal smooth muscle contraction. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endocrine, developmental, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, amniotic fluid, breast milk, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 150 as residues: Pro-36 to Gly-42, Pro-64 to Ala-76, Gly-83 to Ala-90, Ser-100 to Cys-108, Thr-126 to Ser-135. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution primarily in pituitary cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of disorders, particularly of the endocrine system.

Elevated expression of this gene product in the pituitary indicates that it may be possibly a hormone-like substance that either controls pituitary development itself, or various processes controlled by the pituitary. These include growth, milk secretion, smooth muscle contraction, diuresis, blood pressure, and homeostasis. Thus, this gene product may have numerous clinical applications.

Moreover, expression of this gene product in other regions of the brain indicates that it may be involved in normal neurological function, and may be useful in the treatment of a variety of neurological disorders. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", and "Binding Activity" sections below, in Example 11, 17, 18, 19, 20 and 27, and elsewhere herein.

Briefly, the protein can be used for the detection, treatment, and/or prevention of Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism), hypothalamus, and testes. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1036 of SEQ ID NO:58, b is an integer of 15 to 1050, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 49

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

PRVRLATPNIWDL SMLFAFISLLV MLPTWWIVSSWL VWGVILFVYLVIRALRL  
 WRTAKLQVTLKKYSVHLED MATNSRAFTNLVRKALRLIQETEVISRGFTLVS  
 AACPFNKAGQHPSQHLIGLRKAVYRTL RANFQAARLATLYMLKNYPLNSES  
 DNVTTYICVVPFKELGLGLSEEQISEEEAHNFTDGFSLPALKVLFQLWVAQSS  
 EFFRRLALLSTANSPPGPLLTPALLPHRILSDVTQGLPHAHSACLEELKRSYE  
 FYRYFETQHQSV PQCLSKTQQKSRELNNVHTAVRSLQLHLKALLNEVILEDE  
 LEKLVCTKETQELVSEAYPILEQKLKLIQPHVQASNNCWEEAISQVDKLLRRN

TDKKGKPELACENPHCTVSTFEAAYSTHCRQRSNPRGAGIRSLCR (SEQ ID NO: 239). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 5 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by 10 the invention.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 7 - 23 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 24 to 390 of this protein has also been determined. Based upon these characteristics, it is 15 believed that the protein product of this gene shares structural features to type Ib membrane proteins.

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

20 This gene is expressed primarily in prostate and placenta and to a lesser extent in pancreatic tumors and hematopoietic cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are 25 not limited to, prostate cancer; pancreatic cancer; prostate dysfunction; hematopoietic disorders; reproductive diseases and/or disorders, and pancreatitis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and 30 immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, prostate, pancreas, placental, vascular, and cancerous and wounded tissues) or bodily fluids

(e.g., lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 151 as residues: Pro-85 to Ser-94, Pro-127 to Thr-136, Glu-154 to Glu-160, Phe-240 to Ser-250, Leu-255 to Leu-265, Leu-341 to Lys-351, Thr-372 to Gly-384. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also  
10 encompassed by the invention.

The tissue distribution in prostate and placental cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of reproductive disorders. Elevated expression of this gene product in the prostate indicates that it may be involved in normal  
15 prostate function, and may be a diagnostic marker for prostate cancer.

Alternately, expression of this gene product in placenta indicates that it may play a role in normal vascular function, and may be involved in such processes as angiogenesis and endothelial cell chemotaxis. Thus, this gene product may be useful in the treatment of myocardial infarction, cancer, ischemia, and diabetic retinopathy.  
20 Expression of this gene product in placenta may also be indicative of fetal health and development. Similarly, expression of this gene product in hematopoietic cells indicates that it may be involved in the proliferation, differentiation, survival, or activation of all hematopoietic cell lineages. Finally, expression of this gene product in pancreatic cancers indicates that it may play a role in cancer in general, or in  
25 pancreatic function.

The secreted protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, and as nutritional supplements. It may also have a very wide range of biological activities. Representative uses are described in the  
30 "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or

induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2519 of SEQ ID NO:59, b is an integer of 15 to 2533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.

## **25 FEATURES OF PROTEIN ENCODED BY GENE NO: 50**

When tested against Jurkat and K562 cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) and ISRE (interferon-sensitive responsive element ) promoter elements, respectively. Thus, it is likely that this gene activates myeloid, leukemia, and to a lesser extent, other immune or hematopoietic cells and tissue cell-types, through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large,

signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells. ISRE is also a promoter element found upstream in many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

10 In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:  
AAPHPLLRPLCLWCPLWPAWPLRGRPRSAWKRWPLPVGPAKLGCSMTTR  
15 QPTAVSWPCWLMSSSLSTACLAWTLTGSLAREATRRARSLSPWNC SARQV  
PPSPPHSGLGRRGWAHCHLT CLLVTQLFRVGRIHPILSLPLVT (SEQ ID NO:  
240). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
20 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by  
25 the invention.

This gene is expressed primarily in brain and placenta.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, vascular diseases; aberrant angiogenesis; neurological disorders;  
30 learning disorders; placental insufficiency; and fetal distress. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular and neurological systems (CNS/PNS), expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, reproductive, 5 vascular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 152 as residues: Met-1 to Thr-7, Glu-36 to Ser-43, Pro-46 to Gly-63. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

15 — — — The tissue distribution in brain and placental cells and tissues, combined with the detected GAS and ISRE biological activities, indicates that the protein products of this gene are useful for the diagnosis and/or treatment of a variety of neural, reproductive, and vascular diseases and/or disorders. neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are 20 described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, 25 congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the 30 brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Expression of this gene product



in placenta indicates that it may play a role in blood vessel development or function, as the placenta is a highly vascularized organ. Thus, this gene product may be involved in such processes as angiogenesis, endothelial cell chemotaxis, and vascular cord formation. Thus, it may be useful in the treatment of such conditions as myocardial infarction; ischemia; and cancer.

Alternately, expression of this gene product in the brain indicates that it may play a role in the survival, proliferation, or function of neurons, and thus may be useful in the diagnosis and treatment of such neurological disorders as ALS, schizophrenia, and Alzheimer's disease. It may likewise be involved in learning disorders as well. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:60 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 885 of SEQ ID NO:60, b is an integer of 15 to 899, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 51

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

LQLASQSAGIKGMSHCARPTFLTLLASCFWAAAIPNRNVILSVSFRPLHMQ  
FTLSILVFILRILILLRSFL (SEQ ID NO: 241). Polynucleotides encoding these

polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 40 - 56 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 57 to 60 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in spleen derived from patients with chronic lymphocytic leukemia.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, chronic lymphocytic leukemia; hematopoietic disorders; impaired immune function; cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in spleen cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the

diagnosis and/or treatment of a variety of hematopoietic disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Elevated expression of this protein in the spleens of patients with CLL indicates that it may be a useful marker for this disease. Alternately, it may be associated with the development and/or progression of the disease, and may be a useful target for therapeutic intervention. Additionally, this gene product may play more general roles in hematopoiesis, and may serve to control cellular decisions regarding proliferation, survival, activation, and/or differentiation of all hematopoietic cell lineages. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:61 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1065 of SEQ ID NO:61, b is an integer of 15 to 1079, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 52

The translation product of this gene shares sequence homology with a putative protein tyrosine kinase from the Chilo iridescent virus. See, for example, Genbank accession no. gi|2738451 (AF003534). Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with tyrosine kinases and signaling proteins. Such activities are known in the art, some of which are described elsewhere herein.

This gene is expressed in a variety of tissues, including microvascular endothelial cells, dendritic cells, and fetal tissues. as well as several tumors.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, vascular, immune, and developmental diseases and/or disorders, particularly cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., vascular, immune, developmental, proliferative, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, amniotic fluid, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 154 as residues: Ala-21 to Lys-31, Arg-41 to Cys-56, Thr-92 to Cys-102, Arg-132 to Val-137, Lys-152 to Ile-159, Pro-199 to Ser-205, Arg-210 to Asp-219, Ser-225 to Lys-230, Tyr-236 to Ala-241, Lys-243 to Leu-249, Thr-375 to Asp-381. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution and homology to a tyrosine kinase indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis

and treatment of cancer. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of

5 hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for

10 immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders,

15 such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to

20 sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke,

25 atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the

30 endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a

nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:62 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1914 of SEQ ID NO:62, b is an integer of 15 to 1928, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 53**

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 2 - 18 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

20 The translation product of this gene shares some homology with IL-6DPB, a nuclear, leucine-zipper containing, transcriptional regulator protein involved in interleukin-6 signal transduction (see e.g., GenBank Accession GI:204918; all references available through this accession are hereby incorporated herein by reference); for example, Roll, V. et al., Cell 63, 643-653 (1990). Therefore, this gene product is expected to have at least some biological activities in common with transcriptional regulatory proteins.

This gene is expressed primarily in neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders, particularly cancer and immune suppression. Similarly, polypeptides and antibodies directed to

these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 155 as residues: Gly-63 to Ser-72. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in neutrophils indicates that polynucleotides and  
15 polypeptides corresponding to this gene are useful as a marker for neutrophil monitoring in cancer and/or immune suppressed patients and/or during chemotherapy or radiation therapy. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in  
20 regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene  
25 product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues,  
30 such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's

disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:63 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 767 of SEQ ID NO:63, b is an integer of 15 to 781, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 54

This gene is expressed primarily in IL-1 and LPS induced neutrophils, and to a lesser extent, in fetal brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune, hematopoietic, and neural diseases and/or disorders, particularly cancer and immune suppression. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene



at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, amniotic fluid, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 156 as residues: Ile-28 to Trp-37, Ser-68 to Lys-81. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in neutrophils indicates that polynucleotides and polypeptides corresponding to this gene are useful as a marker in neutrophils to monitor patients who are immune suppressed or cancer patients during chemotherapy or radiation therapy. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:64 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1180 of SEQ ID NO:64, b is an integer of 15 to 1194, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.

## **25 FEATURES OF PROTEIN ENCODED BY GENE NO: 55**

This gene is expressed primarily in prostate.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, urogenital diseases and/or disorders, particularly prostate cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the urogenital system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., urogenital, prostate, renal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 157 as residues: Arg-30 to Gln-36. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in prostate cancer cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for study, treatment and diagnosis of prostate cancer and other urogenital disorders. Moreover, the expression within cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection,

treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

- 5 Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

- 10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:65 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is
- 15 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1663 of SEQ ID NO:65, b is an integer of 15 to 1677, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.

20

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 56**

A preferred polypeptide of the invention comprises the following amino acid sequence:

- MVLVLRHPLCARERAFREPGRGLLTRTGQHDGAPAVTAVPGPLGAVAAAEAG  
25 RRSAWGAGGSSPPRKVLWGDMRGRRAGVDVLGPALSSEAAGAEARGWGM  
PGMGVGVGASETRGALFLGREGVHGPCPMDGLGPWPWGPW (SEQ ID NO:  
242). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
30 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the

invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in rejected kidney.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases and/or disorders affecting the kidney. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological  
10 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the urinary tract, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., urogenital, renal, kidney, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal  
15 fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 158 as residues: Ala-30 to Gly-36,  
20 Asp-45 to Trp-50, Lys-65 to Cys-71, Pro-80 to Cys-87. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in kidney indicates the protein product of this gene could be used in the treatment and/or detection of kidney diseases including renal  
25 failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. The protein is useful for modulating the immune response to aberrant  
30 proteins, as may exist in proliferating cells and tissues. Such modulation of the immune response would also show utility in inhibiting the rejection of transplanted tissues, particularly of the renal system. Furthermore, the protein may also be used to

determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the  
 5 above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:66 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
 10 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1223 of SEQ ID NO:66, b is an integer of 15 to 1237, where both a and b correspond to the positions of nucleotide  
 15 residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 57

The translation product of this gene shares sequence homology with both human and mouse Fibulin polypeptides which are extracellular matrix proteins  
 20 found in heart tissue (See Genbank Accession Nos. emb|CAA57876.1 and emb|CAA53040.1, respectively; all references available through these accessions are hereby incorporated herein by reference; for example, J. Cell Biol. 123 (5), 1269-1277 (1993)).

Preferred polypeptides encoded by this gene comprise the following amino  
 25 acid sequence:

MGPAVKMWTNAWKGLDDCHYNQLCENTPGGHRCS CPRGYRMQGPSLPCL  
 DVNECLQLPKACAYQCHNLQGSYRCLCPPGQTLLRDGKACTSLERNQNV  
 TVSHRGPLLPLRPWASIPGTSYHAWVSLRPGPMALSSVGRAWCPPGFIRQN  
 GVCTDLDECRVRNLCQHACRNTEGSYQCLCPAGYRLPSGKNCQDINECEE  
 30 SIECGPGQMCFNTRGSYQCVDTPCPATYRQGSPGTCFRRCSQDCGTGGPSTL  
 QYRLLPLPLGVRAHHDVARLTAFSEVGVPANRTELSMLEPDPRSPFALRPLRA  
 GLGAVYTRRALTRAGLYRLTVRAAAPRHQS FVLLIAVSPYPY (SEQ ID NO:

243). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 10       A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

MRVLVVTIAPIYWALARESGEALNGHSLTGGKFRQSHTWSLLQGAAHDDPV  
ARGLDPDGLLLLDVVVNGVVPGRWLTLQIFKCTRLKKHYVQTRAWPAVRG  
LHTALLPGRPPLVPTLQPQHPVQRGPGPPAPAGAAPAGLSYQLGL (SEQ ID

- 15 NO: 244). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 25       In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

- 30       HASGAFLVVRGEPQGSWGSMTGVINGRKFVATLNTSVMQEAHSGVSSIHSS  
IRHVPANVGPLMRVLVVTIAPIYWALARESGEALNGHSLTGGKFRQESHVEF  
ATGELLTMTQWPGVWIPMASCSTWWSMALSPDSLADADLQVQDFEEHYV  
QTGPGQLFVGSTQRFFQGGLPSFLRCNHSIQYNAARGPQPQLVQHRLRASAISS  
AFDPEAEALRFQLATALQAEENEVGCPEGFELDSQGAFCVDVDECAWDAHL

CREGQRCVNLLGSYRCLPDCGPGFRVADGAGCEDVDECLEGLDDCHYNQLC  
ENTPGGHRCS CPRGYRMQGPSLPCLDVNECLQLPKACAYQCHNLQGSYRCL  
CPPGQTLLRDGKACTSLERNQGNVTTVSHRGPLLWLRPWASIPGTSYHAWV  
SLRPGPMALSSVGRAWCPPGFIRQNGVCTDLDECRVRNLCQHACRNTEGSY  
5 QCLCPAGYRLLPSGKNCQDINECEEESIECGPGQMCFNTRGSYQCVDTPCPAT  
YRQGSPSGTCFRRCSQDCGTGGPSTLQYRLLPLPLGVRAHHDVARLTAFSEV  
GVPANRTELSMLEPDPRSPFALRPLRAGLGAVYTRRALTRAGLYRLTVRAAA  
PRHQSVPVLL IAVSPYPY (SEQ ID NO: 245). Polynucleotides encoding these  
polypeptides are also encompassed by the invention. Moreover, fragments and  
10 variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
complement thereof are encompassed by the invention. Antibodies that bind  
15 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

When tested against U937 and Jurkat cell lines, supernatants removed from  
cells containing this gene repeatedly activated the GAS (gamma activating sequence)  
promoter element. Thus, it is likely that this gene activates myeloid, T-cells, and to a  
20 lesser extent, other immune and hematopoietic cells and tissue cell types, through the  
JAK-STAT signal transduction pathway. GAS is a promoter element found upstream  
of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway  
is a large, signal transduction pathway involved in the differentiation and proliferation  
of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of  
25 the GAS element, can be used to indicate proteins involved in the proliferation and  
differentiation of cells.

This gene is expressed primarily in kidney.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
30 biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, diseases and/or disorders affecting the kidney and renal system.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in



providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the urinary tract, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., renal, urogenital, kidney, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 159 as residues: Lys-32 to Ser-37, His-89 to Gly-94, Asn-124 to Gln-130, Ala-163 to Val-168, Cys-196 to Arg-201, Gln-244 to Gln-264, His-288 to Tyr-294, Leu-314 to Gln-319, Ala-392 to Ser-399, Pro-412 to Asp-419, Ala-452 to Pro-460, Arg-466 to Thr-473. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in rejected kidney, the homology to the conserved Fibulin-2 protein, in addition to the detected GAS biological activity, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of disorders affecting kidneys, particularly proliferative disorders. Representative uses are described here and elsewhere herein.

The protein product of this gene could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:67 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1920 of SEQ ID NO:67, b is an integer of 15 to 1934, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 58

Preferred polypeptides encoded by this gene comprise the following amino acid sequence:

15 MGEKFLLLAMKENHPECFCCKILKILHCMDPGEWLPQTEHCVHLTPKEFLIWT  
MDIASNERSEIQSVALRLASKVISHHMQTCVENRELIAAELKQWVQLVILSCE  
DHLPTESRLAVVEVLTSTTPLFLTNPPILELQDTLALWKCVLTLQSEEQAV  
RDAATETVTTAMSQENTCQSTEFACQVDASIALALALAVLCDLLQQWDQL  
APGLPILLGWLLGESDDLVACVESMHQVEEDYLFKAENVNFWAETLIFVKYL  
20 CKHLFCLLSKSGWRPPSPEMLCHLQRMVSEQCHLLSQFFRELPPAAEFVKTV  
EFTRLRIQEERTLACLRLLAFLEGKEGEDTLVLSVWDSYAESRQLTLPRTEAA  
C (SEQ ID NO: 246). Polynucleotides encoding these polypeptides are also

encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

MGEPNRHPSMFLLLLVLRLYASPMDGTSSALSMGPFVVPFIMRCGHSPVYHS  
REMAARALVPFVMIDHIPNTIRTLSTLPSCDQCFAKPHSWGHSRFFHLL  
QAYSDSKTRNEFRLPARAD (SEQ ID NO: 247). Polynucleotides encoding these  
polypeptides are also encompassed by the invention. Moreover, fragments and  
5 variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides, or the  
complement thereof are encompassed by the invention. Antibodies that bind  
10 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

In another embodiment, polypeptides comprising the amino acid sequence of  
the open reading frame upstream of the predicted signal peptide are contemplated by  
the present invention. Specifically, polypeptides of the invention comprise the  
15 following amino acid sequence:

MTGREFFSRFPELYPFLKQLETVANTVDSMGEPNRHPSMFLLLLVLRLY  
ASPMDGTSSALSMGPFVVPFIMRCGHSPVYHSREMAARALVPFVMIDHIPNTIR  
TLLSTLPSCDQCFAKPHSWGHSRFFHLLQAYSDSKHGTNSDFQHELTDTV  
CTKAKLWLAKRQNPCLVTRAVYIDILFLTCLNRSÄKDNQPVLESIGFWEE  
20 VRGIISGSELITGFPWAFKVPGLPQYLQSLTRLAIAAVWAAAKSGERETNPVI  
SFSQLLESAPFEVRSLEALLEKFLAAASGLGEKGVPLLNCNMGEKFLLLAM  
KENHPECFCILKILHCMDPGEWLPQTEHCVHLTPKEFLIWTMDIASNERSEIQ  
SVALRLASKVISHMQTCVENRELIAAELKQWVQLVILSCEDHLPTE SRLAVV  
EVLSTSTPLFLTNPHPILELQDTLALWKCVLTLQSEEQAVRDAATETVTTAM  
25 SQENTCQSTEFACQVDASIALALAVLCDLLQQWDQLAPGLPILLGWLLG  
ESDDLVA CVESMHQVEEDYLFKA EVNFWAETLIFVKYLCKHLFCLLSKSG  
WRPPSPEMLCHLQRMVSEQCHLLSQFFRELPPAAEFVKTVFETRLRIQEERTL  
ACLRLLAFLEGKEGEDTLVLSVWDSYAESRQLTLPRTEAAC (SEQ ID NO:  
248). Polynucleotides encoding these polypeptides are also encompassed by the  
30 invention. Moreover, fragments and variants of these polypeptides (such as, for  
example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by

the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have two transmembrane domains at about amino acid position 144 - 160, and 462 - 478 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

Included in this invention as a preferred domain is the formate and nitrite transporters domain, which was identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). A number of bacterial and archaeobacterial proteins involved in transporting formate or nitrite have been shown [1] to be related: - *focA* and *focB*, from *Escherichia coli*, transporters involved in the bidirectional transport of formate. - *fdhC*, from *Methanobacterium formicicum* and *thermoformicicum*, a probable formate transporter. - *nirC*, from *Escherichia coli* and *Salmonella typhimurium*, a probable nitrite transporter. - *Bacillus subtilis* hypothetical protein *yrhG*. - *Bacillus subtilis* hypothetical protein *ywcJ* (*ipa-48R*). These transporters are proteins of about 280 residues and seem to contain six transmembrane regions. As signature patterns, we selected two conserved regions. The first one is located in what seems to be a cytoplasmic loop between the second and third transmembrane domains; the second is part of the fourth transmembrane region. The 70 Kd yeast hypothetical protein YHL008c is highly similar, in its N-terminal section, to the prokaryotic members of this family. The consensus pattern is as follows: [LIVMA]-[LIVMY]-x-G-[GSTA]-[DES]-L-[FI]-[TN]-[GS].

Preferred polypeptides of the invention comprise the following amino acid sequence: IISGSELITG (SEQ ID NO: 249). Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further preferred are polypeptides comprising the formate and nitrite transporter domain of the sequence referenced in Table 1 for this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of this

referenced sequence. The additional contiguous amino acid residues may be N-terminal or C-terminal to the formate and nitrite transporter domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the formate and nitrite transporter domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to formate and nitrite transporter proteins. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with formate and nitrite transporter proteins. Such activities are known in the art, some of which are described elsewhere herein.

It is believed that this gene maps to chromosome 2. Accordingly, polynucleotides derived from this gene are useful in linkage analysis as markers for chromosome 2.

This gene is expressed primarily in cells of the immune system, primarily T-cells and to a lesser extent in spleen, liver, thymus, tonsils, and testis.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders, particularly disorders affecting hematopoiesis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells; particularly of hematopoietic cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 160 as residues: Gly-2 to Pro-8, Ser-82 to His-92, Tyr-107 to Asp-117, Arg-162 to Pro-169, Ser-224 to Thr-229, Leu-310 to

His-315, Ser-333 to Glu-338, Glu-381 to Ser-388, Gln-428 to Ala-433, Met-446 to Thr-455, Ser-548 to Ser-554, Gly-613 to Asp-618, Ser-627 to Gln-633.

Polynucleotides encoding said polypeptides are also encompassed by the invention.

Antibodies that bind said polypeptides are also encompassed by the invention.

- 5           The tissue distribution in immune cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of disorders affecting hematopoiesis, including cancers. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein.
- 10       Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the
- 15       gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated
- 20       cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.
- 25           Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used
- 30       to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the

protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:68 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3286 of SEQ ID NO:68, b is an integer of 15 to 3300, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 59

This gene is expressed primarily in bone marrow, CD34 positive cells, and immune cells, including, neutrophils, T-cells, B-cells, macrophages, monocytes, and dendritic cells and to a lesser extent in brain and tonsils.

In one embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention may comprise, or alternatively consist of the following amino acid sequence:

VDGIDKLDIEFLQQFLETHSRGPRLHSPGHASQEATPGANMSSGTELLWPGAA  
LLVLLGVAASLCVRCSRPGAKRSEKIYQQRSLREDQQSFTGSRTYSLVGQAW  
PGPLADMAPTRKDKLLQFYPSLEDPASSRYQNFSKGSRHGSEEAYIDPIAMEY  
YNWGRFSKPPEDDDANSYENVLICKQKTTETGAQQEGIGGLCRGDLSSLAL  
KTGPTSGLCPSASPEEDEGI (SEQ ID NO: 250). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind

polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments may comprise or alternatively consist of one, two, three, four or more of the following amino acid sequence:

- 5 ASSRYQNFSKGSRHGSEEAYIDPIA (SEQ ID NO: 251),  
MEYYNWGRFSKPPEDDDANSY (SEQ ID NO: 252),  
ENVLICKQKTTETGAQQEGIGGLCRGD (SEQ ID NO: 253), VRCSR  
PGAKRSEKIYQQRSLREDQQSFTGSRTYSLVGQAWPGPLADMAPTRKDKLLQ  
FYPSPLEDPASS (SEQ ID NO: 255) and LSLSLALKTGPTSGLCPSASPEEDEGI  
10 (SEQ ID NO: 254). Polynucleotides encoding these polypeptide fragments (SEQ ID  
NOS:251, 252, 253, 254, and/or 255), polynucleotides that hybridize to the  
complementary strand of these polynucleotides (e.g., under the hybridization  
conditions described herein) are encompassed by the invention, as are the  
polypeptides encoded by these hybridizing polynucleotides. Polynucleotides  
15 encoding these polypeptides are also encompassed by the invention. Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
20 polypeptides, or the complement there of are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

- Preferred polypeptides of the present invention comprise, or alternatively  
25 consist of one, two, three, four, five, or more of the immunogenic epitopes shown in  
SEQ ID NO: 161 as residues: Ser-29 to Thr-57, Pro-74 to Lys-79, Pro-85 to Glu-107,  
Tyr-118 to Tyr-136, Gln-144 to Gln-152, Ala-182 to Glu-188. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

- Also preferred are polypeptides comprising the mature polypeptide which is  
30 predicted to consist of residues 26-190 of the foregoing sequence (SEQ ID NO:161),  
and biologically active fragments of the mature polypeptide (e.g., fragments that  
stimulate the proliferation of bone marrow CD34+ cells).



Figures 1A-B show the nucleotide (SEQ ID NO:69) and deduced amino acid sequence (SEQ ID NO:161) of this polypeptide.

Figure 2 shows an analysis of the amino acid sequence (SEQ ID NO:161).

Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention.

The data presented in Figure 2 are also represented in tabular form in Table 7. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 2, and Table 7: "Res": amino acid residue of SEQ ID NO:161 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO:161 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions. The data representing the structural or functional attributes of the protein set forth in Figure 2 and/or Table 7, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment,

the data presented in columns VIII, IX, XIII, and XIV of Table 7 can be used to determine regions of the protein which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figure 2, but may, as shown in Table 7, be represented or identified by using tabular representations of the data presented in Figure 2. The DNA\*STAR computer algorithm used to generate Figure 2 (set on the original default parameters) was used to present the data in Figure 2 in a tabular format (See Table 7). The tabular format of the data in Figure 2 is used to easily determine specific boundaries of a preferred region. The above-mentioned preferred regions set out in Figure 2 and in Table 7 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figures 1A-B (SEQ ID NO:161). As set out in Figure 2 and in Table 7, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and Hopp-Woods hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Jameson-Wolf regions of high antigenic index and Emini surface-forming regions.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated DNA molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in SEQ ID NO:69 is intended DNA fragments at least about 15nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-1500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in SEQ ID NO:69. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the

deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:69. In this context "about" includes the particularly recited size, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Representative examples of polynucleotide fragments of the invention include, for example,

5 fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to about 50, from about 51 to about 100, from about 101 to about 150, from about 151 to about 200, from about 201 to about 250, from about 251 to about 300, from about 301 to about 350, from about 351 to about 400, from about 401 to about 450, from about 451 to about 500, and from about 501 to about 550, and from about 551 to

10 about 600, and from about 601 to about 650, and from about 651 to about 700, and from about 701 to about 750, and from about 751 to about 800, and from about 801 to about 850, and from about 851 to about 900, and from about 901 to about 950, and from about 951 to about 1000, and from about 1001 to about 1050, and from about 1051 to about 1100, and from about 1101 to about 1150, and from

15 about 1151 to about 1200, and from about 1201 to about 1250, and from about 1251 to about 1300, and from about 1301 to about 1350, and from about 1351 to about 1400, and from about 1401 to about 1450, and from about 1451 to about 1500, and from about 1501 to about 1551, and from about 1551 to about 1600, and from about 1601 to about 1650, and from about 1651 to about 1700, and from about 1701 to

20 about 1750, and from about 1751 to about 1797 of SEQ ID NO:69, or the complementary strand thereto, or the cDNA contained in the deposited gene. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In additional embodiments, the polynucleotides of the invention encode functional attributes of the

25 corresponding protein.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-

30 60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant to be a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) or mature-form of the protein. Such functional activities include, but are not limited to, biological activity (e.g., ability to regulate (e.g., stimulate) hematopoiesis *in vitro* or *in vivo*), antigenicity, and immunogenicity. The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods described herein.

In addition, assays described herein and otherwise known in the art may routinely be applied to measure biological activity of polypeptides and fragments of the invention, variants derivatives and analogs thereof (e.g., to regulate (e.g., to stimulate or inhibit) hematopoiesis *in vitro* or *in vivo*). For example, techniques known in the art (such as for example assaying for thymidine incorporation), may be applied or routinely modified to assay for the ability of the compositions of the invention to inhibit proliferation of hematopoietic cells. Other methods will be known to the skilled artisan and are within the scope of the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, etc.) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or

immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence shown in Figure 1A-B (i.e., SEQ ID NO:69), up to the Glu residue at position number 185 and polynucleotides encoding such polypeptides.

Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-190, where m is an integer from 2 to 184, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:161. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: V-26 to I-190; R-27 to I-190; C-28 to I-190; S-29 to I-190; R-30 to I-190; P-31 to I-190; G-32 to I-190; A-33 to I-190; K-34 to I-190; R-35 to I-190; S-36 to I-190; E-37 to I-190; K-38 to I-190; I-39 to I-190; Y-40 to I-190; Q-41 to I-190; Q-42 to I-190; R-43 to I-190; S-44 to I-190; L-45 to I-190; R-46 to I-190; E-47 to I-190; D-48 to I-190; Q-49 to I-190; Q-50 to I-190; S-51 to I-190; F-52 to I-190; T-53 to I-190; G-54 to I-190; S-55 to I-190; R-56 to I-190; T-57 to I-190; Y-58 to I-190; S-59 to I-190; L-60 to I-190; V-61 to I-190; G-62 to I-190; Q-63 to I-190; A-64 to I-190; W-65 to I-190; P-66 to I-190; G-67 to I-190; P-68 to I-190; L-69 to I-190; A-70 to I-190; D-71 to I-190; M-72 to I-190; A-73 to I-190; P-74 to I-190; T-75 to I-190; R-76 to I-190; K-77 to I-190; D-78 to I-190; K-79 to I-190; L-80 to I-190; L-81 to I-190; Q-82 to I-190; F-83 to I-190; Y-84 to I-190; P-85 to I-190; S-86 to I-190; L-87 to I-190; E-88 to I-190; D-89 to I-190; P-90 to I-190; A-91 to I-190; S-92 to I-190; S-93 to I-190; R-94 to I-190; Y-95 to I-190; Q-96 to I-190; N-97 to I-190; F-98 to I-190; S-99 to I-190; K-100 to I-190; G-101 to I-190; S-102 to I-190; R-103 to I-190; H-104 to I-190; G-105 to I-190; S-106 to I-190; E-107 to I-190; E-108 to I-190; A-109 to I-190; Y-110 to I-190; I-111 to I-190; D-112 to I-190; P-113 to I-190; I-114 to I-190; A-115 to I-190; M-116 to I-190; E-117 to I-190; Y-118 to I-190; Y-119 to I-190; N-120 to I-190; W-121 to I-190; G-122 to I-190; R-123 to I-190; F-124 to I-190; S-125 to I-190; K-126 to I-190; P-127 to I-190; P-128 to I-190; E-129 to I-190; D-130 to I-190; D-131 to I-190; D-132 to I-190; A-133 to I-190; N-134 to I-190; S-135 to I-190; Y-136 to I-190; E-137 to I-190; N-138 to I-190; V-139 to I-190; L-140 to I-190; I-141 to I-190; C-142 to I-190;

K-143 to I-190; Q-144 to I-190; K-145 to I-190; T-146 to I-190; T-147 to I-190; E-148 to I-190; T-149 to I-190; G-150 to I-190; A-151 to I-190; Q-152 to I-190; Q-153 to I-190; E-154 to I-190; G-155 to I-190; I-156 to I-190; G-157 to I-190; G-158 to I-190; L-159 to I-190; C-160 to I-190; R-161 to I-190; G-162 to I-190; D-163 to I-190; L-164 to I-190; S-165 to I-190; L-166 to I-190; S-167 to I-190; L-168 to I-190; A-169 to I-190; L-170 to I-190; K-171 to I-190; T-172 to I-190; G-173 to I-190; P-174 to I-190; T-175 to I-190; S-176 to I-190; G-177 to I-190; L-178 to I-190; C-179 to I-190; P-180 to I-190; S-181 to I-190; A-182 to I-190; S-183 to I-190; P-184 to I-190; and E-185 to I-190, of SEQ ID NO:161. Polypeptides encoding these polynucleotides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the

polypeptide shown in Figure 1A-B (SEQ ID NO:161), as described by the general formula 1-n, where n is an integer from 6 to 184 where n corresponds to the position of amino acid residue identified in SEQ ID NO:161. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively

5 consisting of, an amino acid sequence selected from the group: V-26 to G-189; V-26 to E-188; V-26 to D-187; V-26 to E-186; V-26 to E-185; V-26 to P-184; V-26 to S-183; V-26 to A-182; V-26 to S-181; V-26 to P-180; V-26 to C-179; V-26 to L-178; V-26 to G-177; V-26 to S-176; V-26 to T-175; V-26 to P-174; V-26 to G-173; V-26 to T-172; V-26 to K-171; V-26 to L-170; V-26 to A-169; V-26 to L-168; V-26 to S-

10 167; V-26 to L-166; V-26 to S-165; V-26 to L-164; V-26 to D-163; V-26 to G-162; V-26 to R-161; V-26 to C-160; V-26 to L-159; V-26 to G-158; V-26 to G-157; V-26 to I-156; V-26 to G-155; V-26 to E-154; V-26 to Q-153; V-26 to Q-152; V-26 to A-151; V-26 to G-150; V-26 to T-149; V-26 to E-148; V-26 to T-147; V-26 to T-146; V-26 to K-145; V-26 to Q-144; V-26 to K-143; V-26 to C-142; V-26 to I-141; V-26

15 to L-140; V-26 to V-139; V-26 to N-138; V-26 to E-137; V-26 to Y-136; V-26 to S-135; V-26 to N-134; V-26 to A-133; V-26 to D-132; V-26 to D-131; V-26 to D-130; V-26 to E-129; V-26 to P-128; V-26 to P-127; V-26 to K-126; V-26 to S-125; V-26 to F-124; V-26 to R-123; V-26 to G-122; V-26 to W-121; V-26 to N-120; V-26 to Y-119; V-26 to Y-118; V-26 to E-117; V-26 to M-116; V-26 to A-115; V-26 to I-114;

20 V-26 to P-113; V-26 to D-112; V-26 to I-111; V-26 to Y-110; V-26 to A-109; V-26 to E-108; V-26 to E-107; V-26 to S-106; V-26 to G-105; V-26 to H-104; V-26 to R-103; V-26 to S-102; V-26 to G-101; V-26 to K-100; V-26 to S-99; V-26 to F-98; V-26 to N-97; V-26 to Q-96; V-26 to Y-95; V-26 to R-94; V-26 to S-93; V-26 to S-92; V-26 to A-91; V-26 to P-90; V-26 to D-89; V-26 to E-88; V-26 to L-87; V-26 to S-

25 86; V-26 to P-85; V-26 to Y-84; V-26 to F-83; V-26 to Q-82; V-26 to L-81; V-26 to L-80; V-26 to K-79; V-26 to D-78; V-26 to K-77; V-26 to R-76; V-26 to T-75; V-26 to P-74; V-26 to A-73; V-26 to M-72; V-26 to D-71; V-26 to A-70; V-26 to L-69; V-26 to P-68; V-26 to G-67; V-26 to P-66; V-26 to W-65; V-26 to A-64; V-26 to Q-63; V-26 to G-62; V-26 to V-61; V-26 to L-60; V-26 to S-59; V-26 to Y-58; V-26 to T-

30 57; V-26 to R-56; V-26 to S-55; V-26 to G-54; V-26 to T-53; V-26 to F-52; V-26 to S-51; V-26 to Q-50; V-26 to Q-49; V-26 to D-48; V-26 to E-47; V-26 to R-46; V-26 to L-45; V-26 to S-44; V-26 to R-43; V-26 to Q-42; V-26 to Q-41; V-26 to Y-40; V-

26 to I-39; V-26 to K-38; V-26 to E-37; V-26 to S-36; V-26 to R-35; V-26 to K-34; V-26 to A-33; V-26 to G-32; and V-26 to P-31 of SEQ ID NO:161. Polypeptides encoding these polynucleotides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as  
5 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the  
10 invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino  
15 and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:161, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by the cDNA clone contained  
20 in ATCC Deposit No. 209889, where this portion excludes any integer of amino acid residues from 1 to about 184 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209889, or any integer of amino acid residues from 1 to about 190 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy  
25 terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209889. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

The present application is also directed to proteins containing polypeptides at least 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the  
30 polypeptide sequence set forth herein m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and



C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders affecting the immune and hematopoietic systems, particularly hematopoiesis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

This gene has been found to stimulate the proliferation of bone marrow CD34+ cells. This assay which is described in Example 53 herein is based on the ability of human CD34+ to proliferate in presence of hematopoietic growth factors and evaluates the ability of the polypeptides of the invention, and agonists and antagonists thereof, to stimulate or inhibit this proliferation.

The tissue distribution in immune and hematopoietic cells and tissues and the ability to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Moreover, the protein represents a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be

useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof, can also be employed to inhibit the proliferation and  
5 differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

The polynucleotides and/or polypeptides of the invention and/or agonists  
10 and/or antagonists thereof, may also be employed for the expansion of immature hematopoietic progenitor cells, for example, granulocytes, macrophages or monocytes, by temporarily preventing their differentiation. These bone marrow cells may be cultured *in vitro*. Thus, polynucleotides and/or polypeptides of the invention, or agonists or antagonists thereof, may be useful as a modulator of hematopoietic  
15 stem-cells *in vitro* for the purpose of bone marrow transplantation and/or gene therapy. Since stem cells are rare and are most useful for introducing genes into for gene therapy, polynucleotides and/or polypeptides of the invention can be used to isolate enriched populations of stem cells. Stem cells can be enriched by culturing cells in the presence of cytotoxins, such as 5-Fu, which kills rapidly dividing cells,  
20 where as the stem cells will be protected by polynucleotides and/or polypeptides of the invention. These stem cells can be returned to a bone marrow transplant patient or can then be used for transfection of the desired gene for gene therapy. In addition, this gene can be injected into animals which results in the release of stem cells from the bone marrow of the animal into the peripheral blood. These stem cells can be isolated  
25 for the purpose of autologous bone marrow transplantation or manipulation for gene therapy. After the patient has finished chemotherapy or radiation treatment, the isolated stem cells can be returned to the patient.

Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of hematopoietic related disorders such as anemia,  
30 pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone

marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

This gene product may also be involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

This gene may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/ immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Based upon the proteins immune cell specific message distribution, it may be involved in many aspects of the immune response, especially its initial stages, inflammation, allograft rejection,

infectious disease response etc. It is frequently found in the hematopoietic cell cDNA libraries. Thus, this factor could be involved in the control of hematopoietic cell proliferation, differentiation, and function. Based on this one can postulate its use in the management of anemias, leukemias, neutropenia, thrombocytopenia, autoimmune diseases, blood tissue engraftment, and poikilothromerythromatosis. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:69 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1783 of SEQ ID NO:69, b is an integer of 15 to 1797, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 60

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

30 VLWREASALVLSNRLSSGLLHDL L L QPAHSRLFP RRSRGLSEGE GSSVSLQRS  
 RVLSAMKHV L NLYLLGVVLTLLSIFVRVMESLEGLLES PPGT SWTT RSQLAN  
 TEPTKGLPDHPSRSM (SEQ ID NO: 256). Polynucleotides encoding these

polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in immune cells including activated T cells, macrophages, jurkat cells, bone marrow cells, and osteoblasts and to a lesser extent in kidney cortex, brain, placenta and lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders, particularly inflammation and diseases related to inflammatory activity. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 162 as residues: Pro-34 to Met-63. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in immune cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating or diagnosing disease related to the normal or abnormal activation of T cells.

Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:70 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1359 of SEQ ID NO:70, b is an integer of 15 to 1373, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 61

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

YTFHTQIFLDFPMIFLTVLPLAFLFLHSGFYHYISFSLFSLALFFFLDVATFR  
RPGQLFCERSVLFD MFHFGFVSLFLHEWQAKHFWAGLFIVLPSDVFFSVHHL  
EAPDGSFPNIAKLSLILLR (SEQ ID NO: 257). Polynucleotides encoding these

polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have two transmembrane domains at about amino acid position 2 - 18 and 22 - 38 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

This gene is expressed in many tissues including brain, liver, prostate, testes, cartilage, gall bladder. Expression is also seen in a number of tumors including colon carcinoma, pancreas tumor, osteoclastoma, ovarian cancer, B cell lymphoma and acute lymphocytic leukemias. Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell

type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tumors of various organs including the pancreas, colon, and bone. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the major organs, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, hepatic, metabolic, reproductive, testicular, skeletal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, amniotic fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in tumors and proliferative tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating or diagnosing tumors of several major organs including the pancreas and large intestine. This protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.



The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

Furthermore, the protein may also be used to determine biological activity, to raise  
5 antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:71 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
15 — more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1565 of SEQ ID NO:71, b is an integer of 15 to 1579, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.

## 20 **FEATURES OF PROTEIN ENCODED BY GENE NO: 62**

This gene is expressed primarily in dendritic cells and fetal liver/spleen and to a lesser extent in many tissues including tonsils, fetal lung, stromal cell lines, bone marrow cell lines, placenta and tumors including hepatocellular carcinoma, pancreas tumor and osteosarcoma.

25 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases and/or disorders of the immune and hematopoietic system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
30 providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 The tissue distribution in dendritic cells and fetal liver/spleen indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating disorders of the immune system particularly related to the control and generation of precursor cells. Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages.

15 Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein.

Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:72 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1014 of SEQ ID NO:72, b is an integer of 15 to 1028, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 63**

This gene is expressed primarily in adrenal gland tumor and endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endocrine and vascular diseases and/or disorders, particularly diseases associated with the vascular endothelium. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endocrine, vascular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in endothelial cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating disorders that involve the vascular system including diseases such as atherosclerosis, neoangiogenesis associated with tumor growth and conditions associated with inflammation. Moreover, the protein is useful in the detection, treatment, and/or prevention of a variety of vascular disorders and conditions, which include, but are not limited to microvascular disease, vascular leak syndrome, aneurysm, stroke, embolism, thrombosis, coronary artery disease, arteriosclerosis, and/or atherosclerosis. Alternatively, the protein is useful in the treatment, detection, and/or prevention of metabolic disorders, particularly lethargy and depression. Furthermore,

the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:73 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3660 of SEQ ID NO:73, b is an integer of 15 to 3674, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:73, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 64

The translation product of this gene is related to bovine PAM precursor (See, e.g., Genbank accession gi|163482 incorporated herein by reference and also see the following patent publications, which are also incorporated herein by reference in their entirety: J04311386 and WO8902460). Many bioactive peptides terminate with an amino acid alpha-amide at their COOH terminus. The enzyme responsible for this essential posttranslational modification is known as peptidyl-glycine alpha-amidating monooxygenase or PAM. An NH<sub>2</sub>-terminal signal sequence and short propeptide precede the NH<sub>2</sub> terminus of purified PAM. The sequences of several PAM cyanogen bromide peptides were localized in the NH<sub>2</sub>-terminal half of the predicted protein. The forms of PAM purified from bovine neurointermediate pituitary may be generated by endoproteolytic cleavage at a subset of the 10 pairs of basic amino acids in the precursor. High levels of PAM mRNA have been found in bovine pituitary and cerebral cortex. In corticotropic tumor cells, levels of PAM mRNA and pro-ACTH/endorphin mRNA are known to be regulated in parallel by glucocorticoids and CRF.

This gene is expressed primarily in endometrial tumors, dendritic cells, a multiple sclerosis library, kidney, hematopoietic cells, melanocytes, osteoblasts, the spleen, colon, ovary, stromal cells, fetal and adult brain, heart, and in tissues undergoing wound repair.

5           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endometriosis, endometrial cancer, multiple sclerosis, hematopoietic diseases, bone disease, and wound healing. Similarly, polypeptides and antibodies  
10           directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly the hematopoietic system and female reproduction, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, immune,  
15           hematopoieticm integumentary, skeletal, gastrointestinal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, amniotic fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20           The tissue distribution in dendritic and hematopoietic cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful as a therapeutic or diagnostic agent in diseases of hematopoietic origin as well as the female reproductive track due to the gene's primary pattern of expression. Polynucleotides and polypeptides corresponding to this gene are useful for the  
25           treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses  
30           include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as

infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

- 5           The protein may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines;
- 10   immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility);
- 15   chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the
- 20   corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
- 25   related to SEQ ID NO:74 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general
- 30   formula of a-b, where a is any integer between 1 to 2783 of SEQ ID NO:74, b is an integer of 15 to 2797, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 65**

The translation product of this gene shares sequence similarity with several G-protein coupled receptors (See Genbank Accession No. gb|AAC77910.1| (AF061443); all references available through this accession are hereby incorporated herein by reference; for example, Mol. Endocrinol. 12, 1830-1845 (1998)). G-protein coupled receptors are well known in the art and affect a variety of functions.

In particular, the translation product of this gene shares similarity with Follicular Stimulating Hormone Receptor. Preferred polypeptides encoded by this gene comprise the following amino acid sequence:

GTRFPTGETPSLGFTVTLVLLNSLAFLLMAYITKLYCNLEKEDLSENSQSSMI  
KHVAWLIFTNCIFFCPVAFFSFAPLITAISISPEIMKSVTLIFFP (SEQ ID NO:

258). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

25 MIKHVAWLIFTNCIFFCPVAFFSFAPLITAISISPEIMKSVTLIFFPCLLA (SEQ ID NO: 259). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by

the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

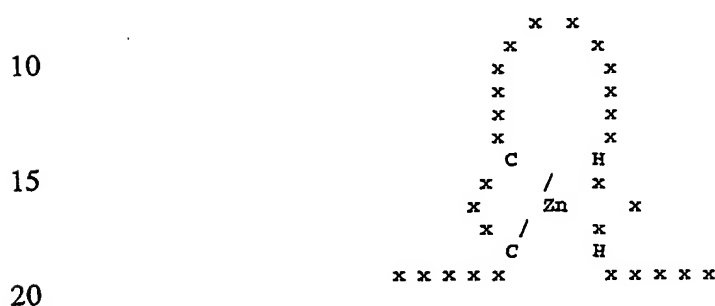
GTRFPTGETPSLGFTVTLVLLNSLAFLLMAVIYTKLYCNLEKEDLSENSQSSMI  
KHVAWLIFTNCIFFCPVAFFSFAPLITAISISPEIMKSVTLIFFPLPACLNPNVLYVF  
FNPKFKEWKLKRRVTKKSGSVSVSISSQGGCLEQDFYYDCGMYSHLQGN  
LTVCDCCESFLLTKPVSCCKHLKSHSCPALAVASCQRPEGYWSDCGTQSAHS  
DYADEEDSFVSDSSDQVQACGRACFYQSRGFPLVRYAYNLPRVKD (SEQ ID  
NO: 260). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 43 - 59 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 60 to 207 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

Included in this invention as preferred domains are Zinc finger, C2H2 type domains, which were identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). 'Zinc finger' domains [1-5] are nucleic acid-binding protein structures first identified in the *Xenopus* transcription factor TFIIIA. These domains have since been found in numerous nucleic acid-binding proteins.



A zinc finger domain is composed of 25 to 30 amino-acid residues. There are two cysteine or histidine residues at both extremities of the domain, which are involved in the tetrahedral coordination of a zinc atom. It has been proposed that such a domain interacts with about five nucleotides. A schematic representation of a zinc finger domain is shown below:



Many classes of zinc fingers are characterized according to the number and positions of the histidine and cysteine residues involved in the zinc atom coordination. In the first class to be characterized, called C2H2, the first pair of zinc coordinating residues are cysteines, while the second pair are histidines. A number of experimental reports have demonstrated the zinc- dependent DNA or RNA binding property of some members of this class. Some of the proteins known to include C2H2-type zinc fingers are listed below. We have indicated, between brackets, the number of zinc finger regions found in each of these proteins; a '+' symbol indicates that only partial sequence data is available and that additional finger domains may be present. In addition to the conserved zinc ligand residues it has been shown that a number of other positions are also important for the structural integrity of the C2H2 zinc fingers. The best conserved position is found four residues after the second cysteine; it is generally an aromatic or aliphatic residue. The consensus pattern is as follows: C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H.

Preferred polypeptides of the invention comprise the following amino acid sequence: CDCCESFLLTKPVSCCKHLIKSH (SEQ ID NO: 261). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as

described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention.

- 5 Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- Further preferred are polypeptides comprising the Zinc finger, C2H2 type domain of the sequence referenced in Table for this gene, and at least 5, 10, 15, 20,  
10 25, 30, 50, or 75 additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues may be N-terminal or C-terminal to the Zinc finger, C2H2 type domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the Zinc finger, C2H2 type domain, wherein the total N- and C-terminal contiguous amino  
15 acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to zinc finger proteins. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with G-coupled proteins, their receptors, and zinc finger proteins. Such activities are known in the art, some of which are described elsewhere herein.
- 20 This gene is expressed primarily in adult and fetal liver, human placenta, colon carcinoma cell lines and fibroblasts and to a lesser extent in the fetal and adult brain, the developing nervous system, lung, pancreas, salivary gland, breast tissue, and dendritic cells.

- Therefore, polynucleotides and polypeptides of the invention are useful as  
25 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the liver, developmental abnormalities, neurologic diseases, lung cancer, pancreatic cancer, and colon cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological  
30 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neurological and hepatic origin, as well as the proliferation and/or differentiation of numerous types of tissues.

expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, immune, hematopoietic, neural, gastrointestinal, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, amniotic fluid, plasma, urine, synovial fluid and spinal fluid) or  
5 another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 167 as residues: Pro-62 to Asp-67,  
10 Arg-74 to Gly-80, Gln-146 to Glu-168. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in fetal liver indicates that polynucleotides and polypeptides corresponding to this gene are useful for a diagnostic marker or  
15 therapeutic in a wide variety of disease states. Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. Representative uses are described in the "Immune Activity" and "Infectious  
20 Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy,  
25 immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, the protein expression in placental and brain tissue indicates the protein is useful in the detection, treatment, and/or prevention of vascular conditions,  
30 which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that

regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. The protein is useful in the treatment, detection, and/or prevention of bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:75 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2689 of SEQ ID NO:75, b is an integer of 15 to 2703, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:75, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 66**

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

ALENSGSPGLQDSARAHFNXSLRSFSFLRNQMYIFELSLYLEGTSFVVVLLFLL  
ISVSLDSPPTTKGWDS VLHIWVPLIVQ (SEQ ID NO: 262). Polynucleotides  
encoding these polypeptides are also encompassed by the invention. Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
5 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides, or the complement thereof are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
10 invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

This gene is expressed primarily in placenta and in hematopoietic cells,  
especially those of T-cell and monocyte origin and to a lesser extent in the brain,  
endothelial cells, and the lungs.

15 ————— Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, hematopoietic, vascular, and developmental diseases and/or disorders.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
20 providing immunological probes for differential identification of the tissue(s) or cell  
type(s). For a number of disorders of the above tissues or cells, particularly of the  
immune system, expression of this gene at significantly higher or lower levels may be  
routinely detected in certain tissues or cell types (e.g., vascular, immune,  
hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
25 serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
taken from an individual having such a disorder, relative to the standard gene  
expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the  
30 immunogenic epitopes shown in SEQ ID NO: 168 as residues: Ser-30 to Trp-37.  
Polynucleotides encoding said polypeptides are also encompassed by the invention.  
Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in hematopoietic cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for therapeutic and/or diagnostic intervention in hematopoietic and developmental disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:76 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 728 of SEQ ID NO:76, b is an

integer of 15 to 742, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 67**

5           This gene is expressed primarily in the prostate and to a lesser extent in human B-cell lymphomas.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
10   not limited to, prostate cancer and diseases of hematopoietic origin, particularly of B-cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate and immune systems, expression of this gene at significantly higher or lower  
15   levels may be routinely detected in certain tissues or cell types (e.g., prostate, reproductive, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, seminal fluid, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or  
20   bodily fluid from an individual not having the disorder.

          Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 169 as residues: Asp-33 to Lys-42. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

25           The tissue distribution in prostate tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful as a therapeutic or diagnostic marker for prostate cancer and disorders involving hematopoietic cells, especially those of B-cell origin. Moreover, the expression within cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular  
30   division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders"

and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. The protein is useful in modulating the immune response to aberrant proteins and polypeptides, as may exist in rapidly proliferating cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:77 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1811 of SEQ ID NO:77, b is an



integer of 15 to 1825, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:77, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 68**

5           When tested against U937 cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates myeloid cells through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

15           In another embodiment, polypeptides comprising the amino acid sequence of the open-reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

GHESICGSCRSWIYFSIRCRRRMRPWWSLLLEACATCAQTGPTRSTSCTQEVSHSSSTAYPAPMRRRCCL PSPRSCT (SEQ ID NO: 263). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

25           The gene encoding the disclosed cDNA is believed to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

This gene is expressed primarily in the brain and the developing embryo and to a lesser extent in the heart, colon, adipose tissue, kidney, mammary tissue, activated T-cells and dendritic cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological diseases, developmental conditions, colon cancer, and hematopoietic diseases, especially of T-cell origin. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, cardiovascular, adipose, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 170 as residues: Thr-18 to Cys-26, Glu-29 to Thr-36, Ser-50 to Thr-55. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in brain, combined with the detected GAS biological activity, indicates that polynucleotides and polypeptides corresponding to this gene are useful for therapeutic and/or diagnostic agents in neurological diseases, developmental abnormalities, colon cancer, and hematopoietic diseases, especially those of T-cell origin. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating

diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:78 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1660 of SEQ ID NO:78, b is an integer of 15 to 1674, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:78, and where b is greater than or equal to a + 14.

25

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 69

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 2 - 18 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type II membrane proteins.

30

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by

the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

KRAGVEVGGLVMALAGSVFVLGGVLVLCVERNGEGEMGWPQHLPKSQPLS  
PPVAVRRCSFERSWIDLLVETSSSMVTCRQQVGTPNGMEGRGGGPKTTFPRL

5 QLSGACAVRPEIQWEV (SEQ ID NO: 264). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, 10 under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in activated monocytes, dendritic cells, and in the tonsils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders, particularly 20 leukemia, lymphomas, tumors of hematopoietic origin. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely 25 detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

30 Preferred polypeptides of the present invention comprise one or more of the immunogenic epitopes shown in SEQ ID NO: 171 as residues: Gln-30 to Leu-38, Asn-75 to Thr-86. Polynucleotides encoding said polypeptides are also encompassed

by the invention. Antibodies that bind said polypeptides are also encompassed by the invention.

The tissue distribution in activated monocytes, dendritic cells, and tonsils indicates that polynucleotides and polypeptides corresponding to this gene are useful as a therapeutic and/or diagnostic agent for leukemias, lymphomas, and other diseases associated with cells of hematopoietic origin. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:79 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2177 of SEQ ID NO:79, b is an integer of 15 to 2191, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 70**

When tested against U937 cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates myeloid cells, and to a lesser extent, other immune cells and tissue cell types, through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

This gene is expressed primarily in the placenta, brain, and liver and to a lesser extent in most other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic, neurological, vascular, and developmental diseases and/or disorders, particularly cancers. Similarly, polypeptides and antibodies directed

in providing immunological probes for differential or cell type(s). For a number of disorders of the above the immune and nervous systems, expression of this lower levels may be routinely detected in certain atopoietic, neurological, vascular, developmental, and (s) or bodily fluids (e.g., lymph, serum, amniotic fluid, fluid and spinal fluid) or another tissue or cell sample ng such a disorder, relative to the standard gene expression level in healthy tissue or bodily fluid from an order.

in brain tissue indicates that polynucleotides and o this gene are useful therapeutic and/or diagnostic agent s, particularly those involving the immune and atative uses are described in the "Regeneration" and s" sections below, in Example 11, 15, and 18, and uses include, but are not limited to the detection, of Alzheimer's Disease, Parkinson's Disease, te Syndrome, meningitis, encephalitis, demyelinating aies, neoplasia, trauma, congenital malformations, spinal farction, aneurysms, hemorrhages, schizophrenia, obsessive compulsive disorder, depression, panic disorder, tychoses, autism, and altered behaviors, including bterns, balance, and perception. In addition, elevated ct in regions of the brain indicates it plays a role in ntially, this gene product is involved in synapse l, learning, cognition, homeostasis, or neuronal oreover, the protein is useful in the detection, treatment, of vascular disorders and conditions, which include, but ular disease, vascular leak syndrome, aneurysm, stroke, ary artery disease, arteriosclerosis, and/or the protein may also be used to determine biological s tissue markers, to isolate cognate ligands or receptors,

to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
 5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:80 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
 10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1321 of SEQ ID NO:80, b is an integer of 15 to 1335, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 71**

The translation product of this gene shares sequence homology with the murine Fig1 (interleukin-four induced gene 1) (See Genbank Accession No AAB51353; all references available through this accession are hereby incorporated herein by reference; for example Chu, C.C. and Paul, W.E., Proc. Natl. Acad. Sci.  
 20 U.S.A. 94 (6), 2507-2512 (1997)). which shares homology to the monoamine oxidases, particularly in domains responsible for FAD binding.

Preferred polypeptides encoded by this gene comprise the following amino acid sequence:

QDWKAERSQDPFEKCMQDPDYEQLLKVTILEADNRIGGRIFTYRDQXTGWIG  
 25 ELGAMRMPSSHRLHKLCQGLGLNLTKFTQYDKNTWTEVHEXKLRNYVVEK  
 VPEKLG YALRPQEKGHSPEDIYQMALNQALKDLKALGCRKAMKKFERHTLL  
 EYLLGEGNLSRPVAVQLLDVMSDGGFFYLSFAEALRAXSCLSDRLQYSRIVG  
 GWDLLPRALLSSLSGLVLLNAPVVAMTQGPHDVHVQIETSPARNLKV LKAD  
 VLLTASGPAVKRITFS (SEQ ID NO: 265), and/or  
 30 LPRHMQEALRRLHYVPATKVFLSFRPFWRREEHIEGGHSNTDRPSRMIFYPPP  
 REGALLASYTWSDA AAFAGLSREEALRLALDDVAALHGPVVRQLWDGT  
 GVVKRWAEDQHSQGGFVVQXPALWQTEKDDWTVPYGRIYFAGEHTAYPHG



WVETAVKSALRAAIKINSRKGPASDTASPEGHASDMEGQGHVHGVASSPSH  
DLAKEEGS (SEQ ID NO: 266). Polynucleotides encoding these polypeptides are  
also encompassed by the invention. Moreover, fragments and variants of these  
polypeptides (such as, for example, fragments as described herein, polypeptides at  
5 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
conditions, to the polynucleotide encoding these polypeptides, or the complement  
there of are encompassed by the invention. Antibodies that bind polypeptides of the  
invention are also encompassed by the invention. Polynucleotides encoding these  
10 polypeptides are also encompassed by the invention.

A preferred polypeptide fragment of the invention comprises the following  
amino acid sequence:

MAPLALHLLVLPILLSLVASQDWKAERSQDPFEKCMQDPDYEQLLKVTIL  
EADNRIGGRIFTYRDQXTGWIGELGAMRMPSSHRILHKLCQGLGLNLTKFTQ  
15 YDKNTWTEVHEXKLRNYVVEKVPEKLGALRPQEKGHSPEDIYQMALNQA  
LKDLKALGCRKAMKKFERHTLLEYLLGEGNLSRPAVQLLGDVMSSEGGFFYL  
SFAEALRAXSCLSDRLQYSRIVGGWDLPRALLSSLSGLVLLNAPVVAMTQG  
PHDVHVQIETSPARNLKV LKADVLLTASGPAVKRITFSPRCPATCRRRCGG  
CTTCRPPRCS (SEQ ID NO: 267). Polynucleotides encoding these polypeptides are  
20 also encompassed by the invention. Moreover, fragments and variants of these  
polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
conditions, to the polynucleotide encoding these polypeptides, or the complement  
25 there of are encompassed by the invention. Antibodies that bind polypeptides of the  
invention are also encompassed by the invention. Polynucleotides encoding these  
polypeptides are also encompassed by the invention.

Based on the sequence similarity, the translation product of this gene is  
expected to share at least some biological activities with monoamine oxidases,  
30 disintegrins, metalloproteinases, and apoptosis modulating proteins. Such activities  
are known in the art, some of which are described elsewhere herein. Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 235 - 251 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 252 to 319 of this protein has also been determined. Based upon these characteristics, it is  
5 believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in hematopoietic cells, particularly in dendritic cells, and activated monocytes and to a lesser extent in T-cells, endothelial cells, and cells associated with ulcerative colitis.

10 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, leukemias, lymphomas, and diseases associated with antigen presenting cells, in addition to apoptosis dependant events. Similarly, polypeptides  
15 and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded  
20 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise one or more of the  
25 immunogenic epitopes shown in SEQ ID NO: 173 as residues: Gln-22 to Gln-44, Ala-90 to Gly-95, Lys-137 to Trp-146, Arg-171 to Asp-181, Glu-370 to Ser-380, Asp-447 to Gly-452, Gln-463 to Trp-469, Asn-504 to Ala-510, Asp-512 to His-519, Ala-541 to Val-550, Asn-558 to His-566. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said polypeptides are also  
30 encompassed by the invention.

The tissue distribution immune and hematopoietic cells and tissues, combined with the homology to the murine Fig1 gene indicates that polynucleotides and

polypeptides corresponding to this gene are useful as a therapeutic and/or diagnostic agent for hematopoietic diseases, especially those associated with antigen presenting cells. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere  
5 herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the  
10 gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated  
15 cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that  
20 influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue  
25 markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:81 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1853 of SEQ ID NO:81, b is an  
5 integer of 15 to 1867, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where b is greater than or equal to a + 14.

Table 1

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HISCN02	209878 05/18/98	pSport1	11	1113	1	1113	232	232	103	1	26	27	106
2	HHGDM70	209878 05/18/98	Lambda ZAP II	12	983	102	983	69	69	104	1	57	58	86
3	HHPGO40	209878 05/18/98	Uni-ZAP XR	13	973	1	973	68	68	105	1	37	38	302
3	HHPGO40	209878 05/18/98	Uni-ZAP XR	82	984	1	984	74	74	174	1	37	38	224
4	HAMGG68	209878 05/18/98	pCMVSPORT 3.0	14	1458	1	1458	312	312	106	1	20	21	55
5	HAPOM49	209878 05/18/98	Uni-ZAP XR	15	2005	1	2005	251	251	107	1	22	23	189
5	HAPOM49	209878 05/18/98	Uni-ZAP XR	83	2664	1	2664	448	448	175	1	1	2	123
6	HBGBA69	209878 05/18/98	Uni-ZAP XR	16	943	1	933	62	62	108	1	38	39	60
7	HBJFJ26	209878 05/18/98	Uni-ZAP XR	17	1503	588	1480	290	290	109	1	26	27	128
7	HBJFJ26	209878 05/18/98	Uni-ZAP XR	84	1328	413	1305	591	591	176	1	20	21	59

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
8	HCEDH38	209878 05/18/98	Uni-ZAP XR	18	1512	1	1438	222	222	110	1	26	27	68
9	HDPOJ08	209878 05/18/98	pCMVSPORT 3.0	19	1655	1	1655	159	159	111	1	18	19	122
10	HDPRX82	209878 05/18/98	pCMVSPORT 3.0	20	2525	1	2525	128	128	112	1	32	33	82
11	HELK31	209878 05/18/98	Uni-ZAP XR	21	1396	25	1334	209	209	113	1	29	30	344
11	HCNUA40	97898 02/26/97 209044 05/15/97	pBluescript	85	1342	949	1237	960	960	177	1	33	34	105
12	HFPCX64	209878 05/18/98	Uni-ZAP XR	22	1069	1	1069	181	181	114	1	28	29	181
12	HFPCX64	209878 05/18/98	Uni-ZAP XR	86	1154	84	1154	257	257	178	1	28	29	87
12	HCEBW71	209225 08/28/97	Uni-ZAP XR	87	1197	141	1197	257	257	179	1	28	29	87
13	HFXDO60	209878 05/18/98	Lambda ZAP II	23	1658	1	1658	131	131	115	1	46	47	115
14	HHEPG41	209878 05/18/98	pCMVSPORT 3.0	24	1077	385	1043	514	514	116	1	35	36	70

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
14	HAUAI83	209626 02/12/98	Uni-ZAP XR	88	910	1	886	253	253	180	1	37	38	49
14	HIPAZ83	97958 03/13/97	Uni-ZAP XR	89	1076	398	1076		575	181	1	11	12	23
15	HKGGAH42	209878 05/18/98	pSport1	25	1205	1	1205	143	143	117	1	21	22	63
16	HMIAP86	209878 05/18/98	Uni-ZAP XR	26	1674	13	1674	182	182	118	1	19	20	334
17	HMUAP70	209878 05/18/98	pCMVSPORT 3.0	27	1965	531	1914	183	183	119	1	16	17	221
17	HMUAP70	209878 05/18/98	pCMVSPORT 3.0	90	1842	407	1783	413	413	182	1	25	26	103
17	HAGFY16	97923 03/07/97	Uni-ZAP XR	91	1963	209	1922	251	251	183	1	28	29	198
17	HBMCF37	209683 03/20/98	pBluescript	92	1487	79	1487	170	170	184	1	44	45	70
17	HFLQB16	209641 02/25/98	Uni-ZAP XR	93	1653	394	1637	413	413	185	1	25	26	82
17	HAGFY16	97923 03/07/97	Uni-ZAP XR	94	1830	87	1786	128	128	186	1	26	27	45
18	HRACJ35	209878 05/18/98	pCMVSPORT 3.0	28	1863	8	1863	99	99	120	1	24	25	472

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
18	HAWAZ34	209141 07/09/97	pBluescript SK-	95	1134	472	1132	687	687	187	1			33
19	HTWDE26	209878 05/18/98	pSport1	29	1626	1	1626	68	68	121	1	30	31	167
19	HMHBN40	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	96	1772	69	1772	129	129	188	1	30	31	231
20	HUSIB13	209878 05/18/98	pSport1	30	605	1	605	172	172	122	1	32	33	46
21	HBAFA02	209877 5/18/98	pSport1	31	931	359	931	46	46	123	1	21	22	108
22	H2CBT75	209877 5/18/98	pBluescript SK-	32	1407	1	1407	32	32	124	1	23	24	60
23	HAGDQ42	209877 5/18/98	Uni-ZAP XR	33	1526	1	1526	126	126	125	1	18	19	248
24	HBMCI42	209877 5/18/98	pBluescript	34	1737	41	1580	244	244	126	1	44	45	248
25	HDPBQ71	209877 5/18/98	pCMVSPORT 3.0	35	2242	6	2242	24	24	127	1	33	34	612
25	HDPFQ90	PTA-499 8/11/99	pCMVSPORT 3.0	97	2381	162	2381	165	165	189	1	33	34	456



Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
26	HCEJG71	209877 5/18/98	Uni-ZAP XR	36	2235	2	2235	28	28	128	1	25	26	447
27	HELHL48	209877 5/18/98	Uni-ZAP XR	37	2971	560	2557	629	629	129	1	16	17	291
27	HSKCT36	209580 1/14/98	Uni-ZAP XR	98	1955	1	1955	31	31	190	1	18	19	184
28	HISAQ04	209877 5/18/98	pSport1	38	1163	1	1163	61	61	130	1	21	22	78
29	HJACB89	209877 5/18/98	pBluescript SK-	39	1932	28	1930	95	95	131	1	23	24	333
30	HTECC05	209877 5/18/98	Uni-ZAP XR	40	881	1	881	27	27	132	1	15	16	164
31	HBJLF01	209877 5/18/98	Uni-ZAP XR	41	1932	201	1931	217	217	133	1	46	47	244
32	HBXGP60	209877 5/18/98	ZAP Express	42	1164	1	1164	143	143	134	1	22	23	55
33	HCE5B20	209877 5/18/98	Uni-ZAP XR	43	1105	1	1105	237	237	135	1	25	26	54
34	HCM5Q56	209877 5/18/98	Uni-ZAP XR	44	1262	1	1262	148	148	136	1	19	20	88
35	HCNAH57	209877 5/18/98	Lambda ZAP II	45	517	1	517	35	35	137	1	33	34	61

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
36	HCUEP91	209877 5/18/98	ZAP Express	46	858	2	858	266	266	138	1	20	21	105
37	HDPICJ91	209877 5/18/98	pCMVSPORT 3.0	47	6107	1	6107	131	131	139	1	28	29	51
38	HDPGK25	209877 5/18/98	pCMVSPORT 3.0	48	703	1	703	345	345	140	1	33	34	119
39	HE2DY70	209877 5/18/98	Uni-ZAP XR	49	639	1	639	137	137	141	1	45	46	58
40	HE2NV57	209877 5/18/98	Uni-ZAP XR	50	867	1	867	99	99	142	1	36	37	99
41	HETBR16	209877 5/18/98	Uni-ZAP XR	51	1569	1	1569	161	161	143	1	21	22	64
42	HFXDG13	209877 5/18/98	Lambda ZAP II	52	1196	1	1196	43	43	144	1	37	38	66
43	HFXKY27	209877 5/18/98	Lambda ZAP II	53	945	1	945	44	44	145	1	19	20	58
44	HHPEC09	209877 5/18/98	Uni-ZAP XR	54	488	1	488	71	71	146	1	19	20	55
45	HISAD54	209877 5/18/98	pSport1	55	2860	1	2860	172	172	147	1	19	20	65
46	HJBCY35	209877 5/18/98	pBluescript SK-	56	1559	93	1272	232	232	148	1	23	24	327

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
47	HKAEA19	209877 5/18/98	pCMVSPORT 2.0	57	2064	1	1909	83	83	149	1	21	22	89
48	HKGDL36	209877 5/18/98	pSport1	58	1050	1	1050	55	55	150	1	33	34	148
49	HLDBS43	209877 5/18/98	pCMVSPORT 3.0	59	2533	1	2533	73	73	151	1	26	27	390
50	HLWAD92	209877 5/18/98	pCMVSPORT 3.0	60	899	1	899	197	197	152	1	34	35	98
51	HL YBI15	209877 5/18/98	pSport1	61	1079	1	1079	92	92	153	1	22	23	60
52	HMEJE05	209889 05/22/98	Lambda ZAP II	62	1928	1	1928	25	25	154	1	30	31	392
53	HNGIX55	209889 05/22/98	Uni-ZAP XR	63	781	1	781	121	121	155	1	19	20	74
54	HNHEX30	209889 05/22/98	Uni-ZAP XR	64	1194	1	1194	138	138	156	1	15	16	81
55	HPJBI33	209889 05/22/98	Uni-ZAP XR	65	1677	1	1677	236	236	157	1	31	32	53
56	HRABA80	209889 05/22/98	pCMVSPORT 3.0	66	1237	1	1237	130	130	158	1	28	29	102
57	HRACD80	209889 05/22/98	pCMVSPORT 3.0	67	1934	1	1934	191	191	159	1	16	17	575

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
57	HRACD80	209889 05/22/98	pCMVSPORT 3.0	99	1958	1	1958	191	191	191	1	16	17	146
58	HSLCX03	209889 05/22/98	Uni-ZAP XR	68	3300	984	2729	677	677	160	1	22	23	643
58	HSLCX03	209889 05/22/98	Uni-ZAP XR	100	2444	1	2444	392	392	192	1	22	23	124
59	HT5GJ57	209889 05/22/98	Uni-ZAP XR	69	1797	92	1797	122	122	161	1	25	26	190
60	HTACS42	209889 05/22/98	Uni-ZAP XR	70	1373	1	1373	213	213	162	1	29	30	63
61	HTEKE40	209889 05/22/98	Uni-ZAP XR	71	1579	1	1579	173	173	163	1	47	48	117
62	HTOBX69	209889 05/22/98	Uni-ZAP XR	72	1028	1	1028	28	28	164	1	20	21	42
63	HUVEO77	209889 05/22/98	Uni-ZAP XR	73	3674	1	3674	55	55	165	1	27	28	47
64	H2CBG48	209889 05/22/98	pBluescript SK-	74	2797	1	2797	125	125	166	1	25	26	45
65	H2CBU83	209889 05/22/98	pBluescript SK-	75	2703	1	2703	157	157	167	1	30	31	207
65	H2CBU83	209889 05/22/98	pBluescript SK-	101	2709	1	2709	157	157	193	1	30	31	51

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
66	HAPNY94	209889 05/22/98	Uni-ZAP XR	76	742	1	742	94	94	168	1	29	30	50
67	HBJHZ58	209889 05/22/98	Uni-ZAP XR	77	1825	1	1825	102	102	169	1	29	30	42
68	HCE2B33	209889 05/22/98	Uni-ZAP XR	78	1674	1	1668	67	67	170	1	18	19	55
69	HDPBQ02	209889 05/22/98	pCMVSPORT 3.0	79	2191	291	2191	460	460	171	1	24	25	108
70	HFIYI70	209889 05/22/98	pSPORT1	80	1335	1	1335	43	43	172	1	15	16	50
71	HDPOZ56	209889 05/22/98	pCMVSPORT 3.0	81	1867	415	1867	103	103	173	1	21	22	566
71	HDPOZ56	209889 05/22/98	pCMVSPORT 3.0	102	1722	1	1722	59	59	194	1	21	22	319

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also  
5 hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

10 Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the  
15 actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the  
20 generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.  
25 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

30 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Table 2 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "Clone ID", for a cDNA-clone related to each contig sequence disclosed in Table 1. Column 2, "Library Code(s)" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 4. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

Table 3, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 3 column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was determined by comparison with the Morbid Map, derived from Online Mendelian



Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>).

- 5 If the putative chromosomal location of the Query overlapped with the chromosomal location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 4, column 3, labelled "OMIM Reference(s)."

A key to the OMIM reference identification numbers is provided in Table 5

- 10 Table 4 provides a key to the Library Code disclosed in Table 2. Column 1 provides the Library Code disclosed in Table 2 column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived. Library codes corresponding to diseased Tissues are indicated in column 3 with the word "disease".

- 15 Table 5 provides a key to the OMIM reference identification numbers disclosed in Table 3 column 3. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web  
20 URL: <http://www.ncbi.nlm.nih.gov/omim/>). Column 2 provides diseases associated with the cytologic band disclosed in Table 3 column 2, as determined using the Morbid Map database.

Table 2

Clone ID	Library Codes
HISCN02	H0539
HHGDM70	H0255 H0333 S0116
HHPGO40	H0051 H0059 H0063 H0083 H0252 H0254 H0265 H0370 H0402 H0421 H0423 H0485 H0490 H0518 H0521 H0522 H0539 H0543 H0556 H0559 H0581 H0597 H0620 H0625 H0634 H0635 H0637 H0638 H0686 H0701 H0702 L1290 S0114 S0116 S0134 S0218 S0292
HAMGG68	H0030 H0050 H0052 H0059 H0110 H0170 H0252 H0320 H0485 H0486 H0488 H0555 H0560 H0644 H0670 H0685 L1290 S0222 S0378 S0412 S0418 S6024
HAPOM49	H0083 H0423 H0494 H0510 H0538 H0542 H0551 H0561 H0575 H0593 H0599 H0616 H0632 H0644 H0662 L1290 S0027 S0045 S0380 S0386 S0420 S0474 T0115
HBGBA69	H0031 H0038 H0039 H0046 H0052 H0068 H0081 H0083 H0087 H0150 H0156 H0181 H0253 H0255 H0266 H0309 H0318 H0327 H0370 H0402 H0413 H0423 H0424 H0436 H0445 H0494 H0506 H0509 H0518 H0519 H0521 H0529 H0530 H0545 H0555 H0571 H0578 H0581 H0592 H0604 H0615 H0616 H0617 H0618 H0622 H0638 H0643 H0653 H0657 H0658 H0661 H0663 H0670 H0682 H0684 H0687 L1290 S0036 S0044 S0046 S0116 S0126 S0142 S0196 S0260 S0278 S0282 S0300 S0342 S0358 S0360 S0380 S0418 S0420 S0474 S3014 T0004 T0049
HBJFJ26	H0031 H0123 H0169 H0263 H0265 H0295 H0318 H0331 H0423 H0510 H0521 H0560 H0613 H0615 L1290 S0010 S0026 S0031 S0052 S0366 S0420
HCEDH38	H0052 H0624 L1290 S0051 S0260 S0300
HDPOJ08	H0024 H0071 H0144 H0156 H0179 H0318 H0341 H0373 H0427 H0428 H0445 H0486 H0488 H0494 H0520 H0521 H0522 H0545 H0547 H0574 H0575 H0580 H0586 H0591 H0616 H0619 H0637 H0647 H0660 H0663 H0665 H0667 H0674 L1290 S0003 S0026 S0031 S0052 S0126 S0142 S0194 S0212 S0214 S0276 S0360 S0380 S0426 S0458 S0474
HDPRX82	H0028 H0031 H0038 H0040 H0051 H0085 H0123 H0124 H0144 H0169 H0272 H0328 H0341 H0412 H0445 H0457 H0519 H0520 H0521 H0574 H0580 H0587 H0618 N0006 S0001 S0051 S0114 S0134 S0152 S0192 S0260 S0282 S0356 S0358 T0067
HELK31	H0013 H0024 H0031 H0040 H0065 H0083 H0100 H0165 H0176 H0232 H0265 H0266 H0318 H0435 H0436 H0445 H0543 H0546 H0547 H0551 H0560 H0566 H0617 H0634 H0640 H0641 H0648 H0658 H0661 H0673 H0696 L1290 S0010 S0045 S0144 S0194 S0206 S0260 S0374 S0414 S3014 T0006 T0041 T0067
HFPCX64	H0052 H0244 L1290 S0222
HFXDO60	S0001
HHEPG41	H0009 H0014 H0024 H0031 H0033 H0040 H0041 H0059 H0060 H0063 H0068 H0083 H0086 H0134 H0135 H0150 H0163 H0214 H0252 H0266 H0271 H0309 H0318 H0341 H0424 H0445 H0455 H0484 H0486 H0506 H0543 H0546 H0553 H0561 H0575 H0576 S0045 S0046 S0126 S0144 S0182 S0192 S0210 S0278 S0330 S3012 T0082 T0114
HKGAH42	H0538 S0260
HMIAP86	H0013 H0052 H0486 H0497 H0542 L1290 S0010 S0046 S0222 S0308 S0360 S6028
HMUAP70	H0009 H0012 H0013 H0032 H0038 H0046 H0050 H0052 H0053 H0056 H0059 H0090 H0107 H0123 H0144 H0156 H0165 H0170 H0171 H0181

	H0265 H0327 H0351 H0357 H0413 H0415 H0421 H0422 H0423 H0431 H0436 H0478 H0494 H0509 H0510 H0521 H0529 H0539 H0542 H0543 H0549 H0553 H0556 H0559 H0563 H0574 H0575 H0580 H0581 H0587 H0591 H0593 H0599 H0616 H0617 H0619 H0620 H0622 H0623 H0624 H0635 H0637 H0641 H0643 H0644 H0648 H0657 H0659 H0661 H0663 H0670 H0672 H0673 L1290 S0010 S0026 S0029 S0036 S0038 S0044 S0049 S0114 S0126 S0132 S0134 S0140 S0192 S0206 S0212 S0214 S0222 S0346 S0358 S0360 S0364 S0374 S0378 S0380 S0386 S0388 S0392 S0404 S0432 S6024 S6028 T0039 T0048 T0069 T0112
HRACJ35	H0013 H0040 H0052 H0144 H0156 H0163 H0166 H0171 H0208 H0231 H0244 H0333 H0427 H0441 H0497 H0509 H0521 H0537 H0553 H0555 H0580 H0619 H0632 H0644 H0650 H0652 H0661 H0668 H0673 H0696 L1290 S0002 S0015 S0028 S0040 S0206 S0210 S0212 S0260 S0346 S0358 S0360 S0364 S0426 S0460 T0060
HTWDE26	H0039 H0042 H0046 H0050 H0059 H0081 H0090 H0100 H0159 H0169 H0170 H0231 H0244 H0251 H0263 H0264 H0286 H0318 H0341 H0365 H0375 H0376 H0421 H0422 H0423 H0431 H0436 H0444 H0445 H0478 H0486 H0494 H0506 H0519 H0521 H0522 H0543 H0544 H0547 H0550 H0556 H0560 H0580 H0586 H0591 H0622 H0624 H0628 H0644 H0648 H0651 H0652 H0656 H0657 H0659 H0660 H0661 H0665 H0686 L0022 L1290 S0001 S0002 S0003 S0007 S0010 S0046 S0052 S0132 S0144 S0214 S0222 S0242 S0328 S0346 S0354 S0356 S0358 S0364 S0370 S0374 S0380 S0426 S0428 S0448 S6024 T0041 T0060 T0115
HUSIB13	H0038 H0040 H0041 H0069 H0081 H0090 H0110 H0125 H0134 H0144 H0156 H0179 H0181 H0185 H0196 H0328 H0331 H0351 H0391 H0393 H0409 H0412 H0413 H0421 H0422 H0486 H0509 H0521 H0530 H0542 H0543 H0549 H0574 H0580 H0581 H0586 H0587 H0592 H0597 H0599 H0616 H0624 H0638 H0641 H0647 H0649 H0657 H0658 H0659 H0663 H0670 H0672 H0674 H0682 H0687 H0688 H0689 H0696 L0022 S0003 S0027 S0036 S0045 S0046 S0051 S0116 S0126 S0132 S0194 S0196 S0222 S0276 S0328 S0344 S0354 S0356 S0358 S0360 S0364 S0366 S0374 S0378 S0384 S0408 S0418 S0422 S3012 S3014 S6014 T0042 T0071
HBAFA02	H0333 H0410 H0411 H0435 H0488 H0520 H0522 H0539 H0546 H0556 H0597 H0620 H0622 H0628 H0633 L0022 S0022
H2CBT75	H0024 H0510 H0521 L0022 S0022 T0110
HAGDQ42	H0013 H0038 H0040 H0046 H0052 H0144 H0156 H0170 H0268 H0327 H0328 H0428 H0455 H0486 H0519 H0520 H0525 H0547 H0551 H0553 H0560 H0575 H0638 H0659 H0667 H0688 L0022 S0010 S0026 S0214 S0250 S0330 S0352 S0358 S6024 S6028
HBMCI42	H0179 H0421 H0439 H0457 H0581 H0583 L0022 S0116 S0190 S0344
HDPBQ71	H0013 H0031 H0038 H0090 H0125 H0156 H0163 H0170 H0171 H0208 H0341 H0373 H0375 H0412 H0423 H0427 H0494 H0506 H0510 H0521 H0539 H0542 H0547 H0551 H0556 H0580 H0615 H0616 H0624 H0633 H0645 H0658 H0661 H0662 H0696 L0022 S0002 S0003 S0011 S0028 S0036 S0037 S0116 S0126 S0132 S0194 S0222 S0242 S0250 S0328 S0344 S0354 S0380 S0390 S0392 S0420 S0422 S0444 S6016 T0067
HCEJG71	H0012 H0014 H0024 H0031 H0036 H0038 H0039 H0052 H0083 H0123 H0135 H0208 H0253 H0264 H0270 H0292 H0327 H0370 H0423 H0494 H0506 H0545 H0547 H0581 H0591 H0593 H0596 H0607 H0616 H0618 H0634 H0644 H0650 H0659 H0687 H0690 L0022 S0001 S0002 S0028 S0037 S0040 S0049 S0152 S0192 S0212 S0242 S0250 S0342 S0344 S0356 S0366 S0374 S0420 T0010 T0067
HELHL48	H0009 H0013 H0014 H0015 H0024 H0032 H0036 H0039 H0046 H0051

	H0052 H0063 H0069 H0081 H0135 H0144 H0163 H0188 H0251 H0265 H0266 H0268 H0269 H0284 H0327 H0333 H0370 H0372 H0373 H0393 H0411 H0412 H0413 H0433 H0435 H0436 H0438 H0441 H0455 H0509 H0521 H0542 H0555 H0556 H0580 H0590 H0599 H0615 H0616 H0620 H0622 H0624 H0631 H0632 H0634 H0644 H0696 L0022 S0003 S0010 S0027 S0036 S0037 S0038 S0040 S0045 S0046 S0049 S0051 S0126 S0132 S0150 S0152 S0222 S0250 S0278 S0328 S0356 S0376 S3014 T0006 T0008 T0049 T0067
HISAQ04	H0539 H0662 S0260
HJACB89	H0046 H0100 H0123 H0135 H0144 H0213 H0264 H0428 H0441 H0494 H0544 H0545 H0547 H0619 H0645 L0022 S0002 S0046 S0126 S0242 S0350 S0418 T0041
HTECC05	H0012 H0038 H0136 H0253 H0445 H0616 H0620 L0022 S0222 T0082
HBJLF01	H0012 H0081 H0318 H0445 H0484 H0518 H0521 H0542 H0550 H0556 H0560 H0592 H0620 H0638 H0673 H0674 L0022 S0328 S0344 S0420 S0474
HBXGP60	H0438 H0586 H0648 H0672 H0684 L0022 S0360 S0364
HCE5B20	H0013 H0052
HCMSQ56	H0196
HCNAH57	H0085
HCUEP91	H0402
HDPCJ91	H0013 H0014 H0024 H0026 H0040 H0059 H0069 H0090 H0144 H0170 H0295 H0428 H0478 H0486 H0494 H0506 H0519 H0520 H0539 H0547 H0553 H0560 H0574 H0575 H0616 H0626 H0641 H0644 H0674 H0690 L0022 S0007 S0040 S0045 S0222 S0242 S0280 S0300 S0360 S0388 S0420 S0452
HDPGK25	H0008 H0013 H0024 H0036 H0039 H0050 H0100 H0105 H0118 H0144 H0170 H0196 H0231 H0234 H0251 H0252 H0253 H0255 H0271 H0318 H0331 H0370 H0373 H0393 H0411 H0412 H0413 H0422 H0427 H0428 H0437 H0444 H0453 H0485 H0486 H0494 H0521 H0522 H0546 H0553 H0555 H0559 H0574 H0575 H0579 H0580 H0586 H0590 H0591 H0592 H0595 H0597 H0599 H0600 H0616 H0622 H0624 H0631 H0634 H0635 H0637 H0638 H0641 H0644 H0646 H0662 H0663 H0664 H0672 H0673 H0684 L0022 S0001 S0002 S0003 S0011 S0013 S0022 S0026 S0037 S0040 S0044 S0112 S0126 S0132 S0146 S0152 S0192 S0194 S0208 S0212 S0222 S0250 S0318 S0330 S0358 S0360 S0364 S0366 S0374 S0376 S0380 S0388 S0418 S0432 T0041
HE2DY70	H0170 L0022 S0003 S0278
HE2NV57	H0100 H0170 H0421 H0457 H0645 S0052 S0374 S0412 S0414
HETBR16	H0046 H0110 H0436 H0445 H0597 L0022 S0422
HFXDG13	H0013 H0030 H0032 H0036 H0059 H0305 H0543 H0615 H0619 H0637 H0694 L0022 S0001 S0052 S0114 S0152 S0216 S0428
HFXKY27	H0256 H0257 S0282
HHPEC09	H0046 H0051 H0086 H0087 H0124 H0294 H0333 H0352 H0445 H0494 H0550 H0555 H0618 H0660 H0661 H0672 H0687 L0022 S0002 S0046 S0049 S0126 S0222 S0356 S0360 T0023 T0049
HISAD54	H0052 H0087 H0539 H0545 H0617 L0022 S0222 S0332 S0358
HJBCY35	H0039 H0040 H0052 H0059 H0087 H0100 H0135 H0150 H0170 H0171 H0181 H0253 H0255 H0266 H0272 H0292 H0318 H0333 H0341 H0424 H0427 H0457 H0484 H0543 H0545 H0550 H0559 H0580 H0593 H0599 H0617 H0618 H0620 H0622 H0623 H0650 H0670 H0704 L0022 S0036 S0049 S0152 S0276 S0418 T0041 T0042
HKAEA19	H0032 H0051 H0083 H0156 H0170 H0264 H0266 H0388 H0494 H0506 H0520 H0543 H0545 H0547 H0617 H0657 H0658 H0670 H0687 H0690 L0022 S0026 S0031 S0112 S0116 S0126 S0132 S0242 S0344 S0358

	S0360 S0380 S0384 S6024 T0042
HKGDL36	H0033 H0213 H0295 H0417 H0418 H0424 H0538 H0539 H0549 H0604 H0661 H0663 H0672 H0684 L0022 S0051 S0114 S0222 S0260 S6026 T0006
HLDBS43	H0031 H0038 H0090 H0144 H0266 H0318 H0333 H0383 H0413 H0478 H0486 H0494 H0509 H0518 H0519 H0520 H0521 H0538 H0542 H0543 H0547 H0581 H0587 H0597 H0674 H0704 L0022 L0470 S0013 S0022 S0032 S0045 S0116 S0126 S0152 S0212 S0214 S0250 S0328 S0380 S0418 S0438 S0462 S6028 T0023
HLWAD92	H0012 H0052 H0063 H0253 H0264 H0318 H0370 H0393 H0494 H0506 H0530 H0539 H0545 H0547 H0553 H0555 H0556 H0586 H0587 H0592 H0593 H0617 H0656 H0660 H0661 H0662 H0670 L0470 S0046 S0212 S0222 S0358 T0002 T0010 T0109
HLYBI15	H0445
HMEJE05	H0013 H0014 H0024 H0170 H0266 H0341 H0519 H0521 H0560 H0580 H0591 H0594 H0632 H0637 H0638 H0673 H0690 L0470 S0003 S0028 S0036 S0218 S0420 S0424 S6022 T0114
HNGIX55	S0052
HNHEX30	L0470 S0053 S0216
HPJBI33	S0152
HRABA80	H0555
HRACD80	H0550 H0555 L0470
HSLCX03	H0014 H0046 H0063 H0087 H0231 H0253 H0264 H0280 H0333 H0486 H0520 H0539 H0547 H0549 H0618 H0620 H0650 L0470 S0028 S0040 S0045 S0134 S0218 S0250 S0358 S0390 S0418 S0424
HT5GJ57	H0013 H0090 H0264 H0271 H0284 H0318 H0341 H0431 H0445 H0457 H0521 H0522 H0580 H0584 H0656 H0702 S0002 S0003 S0052 S0142 S0144 S0278
HTACS42	H0031 H0042 H0069 H0083 H0341 H0351 H0399 H0431 H0494 H0518 H0519 H0543 H0634 H0635 H0647 H0658 H0672 L0470 S0007 S0027 S0116 S0126 S0132 S0218 S0222 S0260 S0298 S0338 S0344 S0356 S0360 T0060
HTEKE40	H0014 H0038 H0039 H0144 H0212 H0266 H0318 H0328 H0355 H0423 H0445 H0494 H0518 H0539 H0543 H0581 H0597 H0616 H0624 L0470 S0192 S0214 S0334 T0115
HTOBX69	H0014 H0272 H0331 H0341 H0486 H0494 H0521 H0539 H0551 H0561 H0623 H0640 H0659 H0682 H0686 L0470 S0026 S0152 S0358 S0438 T0040
HUVEO77	H0038 H0051 H0052 H0056 H0100 H0156 H0170 H0196 H0343 H0369 H0400 H0411 H0412 H0427 H0431 H0509 H0520 H0547 H0555 H0575 H0590 H0591 H0592 H0597 H0622 H0623 H0652 H0689 H0696 H0708 L0470 S0031 S0045 S0046 S0050 S0051 S0210 S0222 S0280 S0282 S0330 S0346 S0358 S0360 S0380 S0414 S0472
H2CBG48	H0013 H0046 H0090 H0156 H0540 H0542 H0553 H0555 H0620 H0624 L0470 S0002 S0026 S0126 S0144 S0222 S0308 S0356 S0426 S0458 T0110
H2CBU83	H0013 H0031 H0036 H0123 H0144 H0393 H0478 H0539 H0547 H0571 H0574 H0586 H0593 H0622 H0624 H0656 H0670 L0470 S0242 S0276 S0282 S0358 S0374 S0378 S0380 S0414 S0422 S6028 T0103 T0110
HAPNY94	H0031 H0042 H0069 H0220 H0250 H0264 H0265 H0271 H0300 H0413 H0422 H0455 H0484 H0521 H0542 H0545 H0547 H0556 H0575 H0580 H0581 H0617 H0622 H0634 H0635 H0641 H0643 H0644 H0660 H0688 L0470 S0002 S0046 S0049 S0114 S0216 S0222 S0328 S0356 S0360 S0414
HBJHZ58	H0032 H0318

HCE2B33	H0013 H0052 H0097 H0100 H0144 H0232 H0261 H0333 H0359 H0427 H0521 H0543 H0555 H0556 H0619 H0620 H0650 H0682 L0470 S0049 S0356 S0360
HDPBQ02	H0264 H0521 S0002
HFIYI70	H0024 H0046 H0052 H0171 H0253 H0333 H0352 H0393 H0423 H0433 H0442 H0483 H0520 H0521 H0539 H0542 H0545 H0616 H0624 H0628 H0641 H0650 H0652 H0670 H0698 L0470 S0126 S0150 S0242 S0358 S0426 T0010
HDPOZ56	H0250 H0255 H0423 H0435 H0506 H0521 H0522 H0542 H0543 H0555 H0560 H0617 H0618 H0638 H0641 H0658 H0663 H0689 H0695 L0470 S0046 S0422 S0426

**Table 3**

SEQ ID NO: X	Cytologic Band or Chromosome:	OMIM Reference(s):
26	Xq24	300046 300123 301201 301835 301845 307150 310490 311850
28	8q22.2	148900 216550
30	2p13.3-p13.1	203800 253601
31	20q12-q13	600281
44	1q21.2-q22	104770 107670 110700 145001 146760 146790 159440 186780 191030 191315 600923 601412 601652 601863 602491

Table 4

Library Code	Library Description
H0008	Whole 6 Week Old Embryo
H0009	Human Fetal Brain
H0012	Human Fetal Kidney
H0013	Human 8 Week Whole Embryo
H0014	Human Gall Bladder
H0015	Human Gall Bladder, fraction II
H0024	Human Fetal Lung III
H0026	Namalwa Cells
H0028	Human Old Ovary
H0030	Human Placenta
H0031	Human Placenta
H0032	Human Prostate
H0033	Human Pituitary
H0036	Human Adult Small Intestine
H0038	Human Testes
H0039	Human Pancreas Tumor
H0040	Human Testes Tumor
H0041	Human Fetal Bone
H0042	Human Adult Pulmonary
H0046	Human Endometrial Tumor
H0050	Human Fetal Heart
H0051	Human Hippocampus
H0052	Human Cerebellum
H0053	Human Adult Kidney
H0056	Human Umbilical Vein, Endo. remake
H0059	Human Uterine Cancer
H0060	Human Macrophage
H0063	Human Thymus
H0065	Human Esophagus, Normal
H0068	Human Skin Tumor
H0069	Human Activated T-Cells
H0071	Human Infant Adrenal Gland
H0081	Human Fetal Epithelium (Skin)
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES
H0085	Human Colon
H0086	Human epithelioid sarcoma
H0087	Human Thymus
H0090	Human T-Cell Lymphoma
H0097	Human Adult Heart, subtracted
H0100	Human Whole Six Week Old Embryo
H0105	Human Fetal Heart, subtracted
H0107	Human Infant Adrenal Gland, subtracted
H0110	Human Old Ovary, subtracted
H0118	Human Adult Kidney
H0123	Human Fetal Dura Mater
H0124	Human Rhabdomyosarcoma
H0125	Cem cells cyclohexamide treated
H0134	Raji Cells, cyclohexamide treated



H0135	Human Synovial Sarcoma
H0136	Supt Cells, cyclohexamide treated
H0144	Nine Week Old Early Stage Human
H0150	Human Epididymus
H0156	Human Adrenal Gland Tumor
H0159	Activated T-Cells, 8 hrs., ligation 2
H0163	Human Synovium
H0165	Human Prostate Cancer, Stage B2
H0166	Human Prostate Cancer, Stage B2 fraction
H0169	Human Prostate Cancer, Stage C fraction
H0170	12 Week Old Early Stage Human
H0171	12 Week Old Early Stage Human, II
H0176	CAMA1Ee Cell Line
H0179	Human Neutrophil
H0181	Human Primary Breast Cancer
H0185	Activated T-Cell labeled with 4-thioluri
H0188	Human Normal Breast
H0196	Human Cardiomyopathy, subtracted
H0208	Early Stage Human Lung, subtracted
H0212	Human Prostate, subtracted
H0213	Human Pituitary, subtracted
H0214	Raji cells, cyclohexamide treated, subtracted
H0220	Activated T-Cells, 4 hrs, subtracted
H0231	Human Colon, subtraction
H0232	Human Colon, differential expression
H0234	human colon cancer, metastatic to liver, differentially expressed
H0244	Human 8 Week Whole Embryo, subtracted
H0250	Human Activated Monocytes
H0251	Human Chondrosarcoma
H0252	Human Osteosarcoma
H0253	Human adult testis, large inserts
H0254	Breast Lymph node cDNA library
H0255	breast lymph node CDNA library
H0256	HL-60, unstimulated
H0257	HL-60, PMA 4H
H0261	H. cerebellum, Enzyme subtracted
H0263	human colon cancer
H0264	human tonsils
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco
H0266	Human Microvascular Endothelial Cells, fract. A
H0268	Human Umbilical Vein Endothelial Cells, fract. A
H0269	Human Umbilical Vein Endothelial Cells, fract. B
H0270	HPAS (human pancreas, subtracted)
H0271	Human Neutrophil, Activated
H0272	HUMAN TONSILS, FRACTION 2
H0280	K562 + PMA (36 hrs)
H0284	Human OB MG63 control fraction I
H0286	Human OB MG63 treated (10 nM E2) fraction I
H0292	Human OB HOS treated (10 nM E2) fraction I
H0294	Amniotic Cells - TNF induced
H0295	Amniotic Cells - Primary Culture
H0300	CD34 positive cells (Cord Blood)
H0305	CD34 positive cells (Cord Blood)

H0309	Human Chronic Synovitis
H0318	HUMAN B CELL LYMPHOMA
H0320	Human frontal cortex
H0327	human corpus colosum
H0328	human ovarian cancer
H0331	Hepatocellular Tumor
H0333	Hemangiopericytoma
H0341	Bone Marrow Cell Line (RS4,11)
H0343	stomach cancer (human)
H0351	Glioblastoma
H0352	wilm's tumor
H0355	Human Liver
H0357	H. Normalized Fetal Liver, II
H0359	KMH2 cell line
H0365	Osteoclastoma-normalized B
H0369	H. Atrophic Endometrium
H0370	H. Lymph node breast Cancer
H0372	Human Testes
H0373	Human Heart
H0375	Human Lung
H0376	Human Spleen
H0383	Human Prostate BPH, re-excision
H0388	Human Rejected Kidney, 704 re-excision
H0391	H. Meningioma, M6
H0393	Fetal Liver, subtraction II
H0399	Human Kidney Cortex, re-rescue
H0400	Human Striatum Depression, re-rescue
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision
H0409	H. Striatum Depression, subtracted
H0410	H. Male bladder, adult
H0411	H Female Bladder, Adult
H0412	Human umbilical vein endothelial cells, IL-4 induced
H0413	Human Umbilical Vein Endothelial Cells, uninduced
H0415	H. Ovarian Tumor, II, OV5232
H0417	Human Pituitary, subtracted VIII
H0418	Human Pituitary, subtracted VII
H0421	Human Bone Marrow, re-excision
H0422	T-Cell PHA 16 hrs
H0423	T-Cell PHA 24 hrs
H0424	Human Pituitary, subt IX
H0427	Human Adipose
H0428	Human Ovary
H0431	H. Kidney Medulla, re-excision
H0433	Human Umbilical Vein Endothelial cells, frac B, re-excision
H0435	Ovarian Tumor 10-3-95
H0436	Resting T-Cell Library,II
H0437	H Umbilical Vein Endothelial Cells, frac A, re-excision
H0438	H. Whole Brain #2, re-excision
H0439	Human Eosinophils
H0441	H. Kidney Cortex, subtracted
H0442	H. Striatum Depression, subt II
H0444	Spleen metastatic melanoma
H0445	Spleen, Chronic lymphocytic leukemia

H0453	H. Kidney Pyramid, subtracted
H0455	H. Striatum Depression, subt
H0457	Human Eosinophils
H0478	Salivary Gland, Lib 2
H0483	Breast Cancer cell line, MDA 36
H0484	Breast Cancer Cell line, angiogenic
H0485	Hodgkin's Lymphoma I
H0486	Hodgkin's Lymphoma II
H0488	Human Tonsils, Lib 2
H0490	HL-60, untreated, subtracted
H0494	Keratinocyte
H0497	HEL cell line
H0506	Ulcerative Colitis
H0509	Liver, Hepatoma
H0510	Human Liver, normal
H0518	pBMC stimulated w/ poly I/C
H0519	NTERA2, control
H0520	NTERA2 + retinoic acid, 14 days
H0521	Primary Dendritic Cells, lib 1
H0522	Primary Dendritic cells, frac 2
H0525	PCR, pBMC I/C treated
H0529	Myeloid Progenitor Cell Line
H0530	Human Dermal Endothelial Cells, untreated
H0537	H. Primary Dendritic Cells, lib 3
H0538	Merkel Cells
H0539	Pancreas Islet Cell Tumor
H0540	Skin, burned
H0542	T Cell helper I
H0543	T cell helper II
H0544	Human endometrial stromal cells
H0545	Human endometrial stromal cells-treated with progesterone
H0546	Human endometrial stromal cells-treated with estradiol
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)
H0549	H. Epididymus, caput & corpus
H0550	H. Epididymus, cauda
H0551	Human Thymus Stromal Cells
H0553	Human Placenta
H0555	Rejected Kidney, lib 4
H0556	Activated T-cell(12h)/Thiouridine-re-excision
H0559	HL-60, PMA 4H, re-excision
H0560	KMH2
H0561	L428
H0563	Human Fetal Brain, normalized 50021F
H0566	Human Fetal Brain, normalized c50F
H0571	Human Fetal Brain, normalized C500HE
H0574	Hepatocellular Tumor, re-excision
H0575	Human Adult Pulmonary, re-excision
H0576	Resting T-Cell, re-excision
H0578	Human Fetal Thymus
H0579	Pericardium
H0580	Dendritic cells, pooled
H0581	Human Bone Marrow, treated
H0583	B Cell lymphoma

H0584	Activated T-cells, 24 hrs, re-excision
H0586	Healing groin wound, 6.5 hours post incision
H0587	Healing groin wound, 7.5 hours post incision
H0590	Human adult small intestine, re-excision
H0591	Human T-cell lymphoma, re-excision
H0592	Healing groin wound - zero hr post-incision (control)
H0593	Olfactory epithelium, nasal cavity
H0594	Human Lung Cancer, re-excision
H0595	Stomach cancer (human), re-excision
H0596	Human Colon Cancer, re-excision
H0597	Human Colon, re-excision
H0599	Human Adult Heart, re-excision
H0600	Healing Abdomen wound, 70&90 min post incision
H0604	Human Pituitary, re-excision
H0607	H. Leukocytes, normalized cot 50A3
H0613	H. Leukocytes, normalized cot 5B
H0615	Human Ovarian Cancer Reexcision
H0616	Human Testes, Reexcision
H0617	Human Primary Breast Cancer Reexcision
H0618	Human Adult Testes, Large Inserts, Reexcision
H0619	Fetal Heart
H0620	Human Fetal Kidney, Reexcision
H0622	Human Pancreas Tumor, Reexcision
H0623	Human Umbilical Vein, Reexcision
H0624	12 Week Early Stage Human II, Reexcision
H0625	Ku 812F Basophils Line
H0626	Saos2 Cells, Untreated
H0628	Human Pre-Differentiated Adipocytes
H0631	Saos2, Dexamethasone Treated
H0632	Hepatocellular Tumor, re-excision
H0633	Lung Carcinoma A549 TNFalpha activated
H0634	Human Testes Tumor, re-excision
H0635	Human Activated T-Cells, re-excision
H0637	Dendritic Cells From CD34 Cells
H0638	CD40 activated monocyte dendritic cells
H0640	Ficoll Human Stromal Cells, Untreated
H0641	LPS activated derived dendritic cells
H0643	Hep G2 Cells, PCR library
H0644	Human Placenta (re-excision)
H0645	Fetal Heart, re-excision
H0646	Lung, Cancer (4005313 A3): Invasive Poorly Differentiated Lung Adenocarcinoma,
H0647	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot
H0649	Lung, Normal: (4005313 B1)
H0650	B-Cells
H0651	Ovary, Normal: (9805C040R)
H0652	Lung, Normal: (4005313 B1)
H0653	Stromal Cells
H0656	B-cells (unstimulated)
H0657	B-cells (stimulated)
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma

H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma
H0661	Breast, Cancer: (4004943 A5)
H0662	Breast, Normal: (4005522B2)
H0663	Breast, Cancer: (4005522 A2)
H0664	Breast, Cancer: (9806C012R)
H0665	Stromal cells 3.88
H0667	Stromal cells(HBM3.18)
H0668	stromal cell clone 2.5
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma
H0672	Ovary, Cancer: (4004576 A8)
H0673	Human Prostate Cancer, Stage B2, re-excision
H0674	Human Prostate Cancer, Stage C, re-excision
H0682	Ovarian cancer, Serous Papillary Adenocarcinoma
H0684	Ovarian cancer, Serous Papillary Adenocarcinoma
H0685	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3
H0686	Adenocarcinoma of Ovary, Human Cell Line
H0687	Human normal ovary(#9610G215)
H0688	Human Ovarian Cancer(#9807G017)
H0689	Ovarian Cancer
H0690	Ovarian Cancer, # 9702G001
H0694	Prostate cancer (adenocarcinoma)
H0695	mononucleocytes from patient
H0696	Prostate Adenocarcinoma
H0698	NK Cells Yao20 IL2 treated for 48 hrs
H0701	NK yao15(control)
H0702	NK15(IL2 treated for 48 hours)
H0704	Prostate Adenocarcinoma cell line cultured in vivo in mice
H0708	Human Skeletal Muscle
L0022	Weizmann Olfactory Epithelium
L0470	NCI CGAP Lym12
L1290	Human fetal heart, Lambda ZAP Express
N0006	Human Fetal Brain
S0001	Brain frontal cortex
S0002	Monocyte activated
S0003	Human Osteoclastoma
S0007	Early Stage Human Brain
S0010	Human Amygdala
S0011	STROMAL -OSTEOCLASTOMA
S0013	Prostate
S0015	Kidney medulla
S0022	Human Osteoclastoma Stromal Cells - unamplified
S0026	Stromal cell TF274
S0027	Smooth muscle, serum treated
S0028	Smooth muscle, control
S0029	brain stem
S0031	Spinal cord
S0032	Smooth muscle-ILb induced
S0036	Human Substantia Nigra
S0037	Smooth muscle, IL1b induced
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb
S0040	Adipocytes
S0044	Prostate BPH

S0045	Endothelial cells-control
S0046	Endothelial-induced
S0049	Human Brain, Striatum
S0050	Human Frontal Cortex, Schizophrenia
S0051	Human Hypothalamus, Schizophrenia
S0052	neutrophils control
S0053	Neutrophils IL-1 and LPS induced
S0112	Hypothalamus
S0114	Anergic T-cell
S0116	Bone marrow
S0126	Osteoblasts
S0132	Epithelial-TNF $\alpha$ and INF induced
S0134	Apoptotic T-cell
S0140	eosinophil-IL5 induced
S0142	Macrophage-oxLDL
S0144	Macrophage (GM-CSF treated)
S0146	prostate-edited
S0150	LNCAP prostate cell line
S0152	PC3 Prostate cell line
S0182	Human B Cell 8866
S0190	Prostate BPH, Lib 2, subtracted
S0192	Synovial Fibroblasts (control)
S0194	Synovial hypoxia
S0196	Synovial IL-1/TNF stimulated
S0206	Smooth Muscle- HASTE normalized
S0208	Mesangial cell, frac 1
S0210	Mesangial cell, frac 2
S0212	Bone Marrow Stromal Cell, untreated
S0214	Human Osteoclastoma, re-excision
S0216	Neutrophils IL-1 and LPS induced
S0218	Apoptotic T-cell, re-excision
S0222	H. Frontal cortex, epileptic, re-excision
S0242	Synovial Fibroblasts (III/TNF), subt
S0250	Human Osteoblasts II
S0260	Spinal Cord, re-excision
S0276	Synovial hypoxia-RSF subtracted
S0278	H Macrophage (GM-CSF treated), re-excision
S0280	Human Adipose Tissue, re-excision
S0282	Brain Frontal Cortex, re-excision
S0292	Osteoarthritis (OA-4)
S0298	Bone marrow stroma, treated
S0300	Frontal lobe, dementia, re-excision
S0308	Spleen/normal
S0318	Human Normal Cartilage Fraction II
S0328	Palate carcinoma
S0330	Palate normal
S0332	Pharynx carcinoma
S0334	Human Normal Cartilage Fraction III
S0338	Human Osteoarthritic Cartilage Fraction III
S0342	Adipocytes, re-excision
S0344	Macrophage-oxLDL, re-excision
S0346	Human Amygdala, re-excision
S0350	Pharynx Carcinoma

S0352	Larynx Carcinoma
S0354	Colon Normal II
S0356	Colon Carcinoma
S0358	Colon Normal III
S0360	Colon Tumor II
S0364	Human Quadriceps
S0366	Human Soleus
S0370	Larynx carcinoma II
S0374	Normal colon
S0376	Colon Tumor
S0378	Pancreas normal PCA4 No
S0380	Pancreas Tumor PCA4 Tu
S0384	Tongue carcinoma
S0386	Human Whole Brain, re-excision
S0388	Human Hypothalamus, schizophrenia, re-excision
S0390	Smooth muscle, control, re-excision
S0392	Salivary Gland
S0404	Rectum normal
S0408	Colon, normal
S0412	Temporal cortex-Alzheimer, subtracted
S0414	Hippocampus, Alzheimer Subtracted
S0418	CHME Cell Line, treated 5 hrs
S0420	CHME Cell Line, untreated
S0422	Mo7e Cell Line GM-CSF treated (1ng/ml)
S0424	TF-1 Cell Line GM-CSF Treated
S0426	Monocyte activated, re-excision
S0428	Neutrophils control, re-excision
S0432	Sinus piriformis Tumour
S0438	Liver Normal Met5No
S0444	Colon Tumor
S0448	Larynx Normal
S0452	Thymus
S0458	Thyroid Normal (SDCA2 No)
S0460	Thyroid Tumour
S0462	Thyroid Thyroiditis
S0472	Lung Mesothelium
S0474	Human blood platelets
S3012	Smooth Muscle Serum Treated, Norm
S3014	Smooth muscle, serum induced, re-exc
S6014	H. hypothalamus, frac A
S6016	H. Frontal Cortex, Epileptic
S6022	H. Adipose Tissue
S6024	Alzheimers, spongy change
S6026	Frontal Lobe, Dementia
S6028	Human Manic Depression Tissue
T0002	Activated T-cells
T0004	Human White Fat
T0006	Human Pineal Gland
T0008	Colorectal Tumor
T0010	Human Infant Brain
T0023	Human Pancreatic Carcinoma
T0039	HSA 172 Cells
T0040	HSC172 cells

T0041	Jurkat T-cell G1 phase
T0042	Jurkat T-Cell, S phase
T0048	Human Aortic Endothelium
T0049	Aorta endothelial cells + TNF-a
T0060	Human White Adipose
T0067	Human Thyroid
T0069	Human Uterus, normal
T0071	Human Bone Marrow
T0082	Human Adult Retina
T0103	Human colon carcinoma (HCC) cell line
T0109	Human (HCC) cell line liver (mouse) metastasis, remake
T0110	Human colon carcinoma (HCC) cell line, remake
T0112	Human (Caco-2) cell line, adenocarcinoma, colon
T0114	Human (Caco-2) cell line, adenocarcinoma, colon, remake
T0115	Human Colon Carcinoma (HCC) cell line



Table 5

OMIM ID	OMIM Description
104770	Amyloidosis, secondary, susceptibility to (1)
107670	Apolipoprotein A-II deficiency (3)
110700	Vivax malaria, susceptibility to (1)
145001	Hyperparathyroidism-jaw tumor syndrome (2)
146760	[IgG receptor I, phagocytic, familial deficiency of] (1)
146790	Lupus nephritis, susceptibility to (3)
148900	Klippel-Feil syndrome with laryngeal malformation (2)
159440	Charcot-Marie-Tooth neuropathy-1B, 118200 (3) Dejerine-Sottas disease, myelin P(0)-related, 145900 (3) Hypomyelination, congenital (3)
186780	CD3, zeta chain, deficiency (1)
191030	Nemaline myopathy-1, 161800 (3)
191315	Insensitivity to pain, congenital, with anhidrosis, 256800 (3)
203800	Alstrom syndrome (2)
216550	Cohen syndrome (2)
253601	Miyoshi myopathy, 254130 (2) Muscular dystrophy, limb-girdle, type 2B (2)
300046	Mental retardation, X-linked 23, nonspecific (2)
300123	Mental retardation with isolated growth hormone deficiency (2)
301201	Amelogenesis imperfecta-3, hypoplastic type (2) (?)
301835	Arts syndrome (2)
301845	Bazex syndrome (2)
307150	Hypertrichosis, congenital generalized (2)
310490	Cowchock syndrome (2)
311850	Phosphoribosyl pyrophosphate synthetase-related gout (3)
600281	MODY, type 1, 125850 (3) Non-insulin-dependent diabetes mellitus, 125853 (3)
600923	Porphyria variegata, 176200 (3)
601412	Deafness, autosomal dominant 7 (2)
601652	Glaucoma 1A, primary open angle, juvenile-onset, 137750 (3)
601863	Bare lymphocyte syndrome, complementation group C (1)
602491	Hyperlipidemia, familial combined, 1 (2)

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

### Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature

forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty.

Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

#### **Polynucleotide and Polypeptide Variants**

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the

nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).  
5 Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).  
10

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.  
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As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined  
30

using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

10           If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

25           For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base

subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1,

Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal



ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

5 The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or  
10 added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of  
15 several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

20 Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988  
25 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

30 Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational

analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule  
5 could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or  
10 C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular  
15 polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions,  
20 inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

25 The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these  
30 positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used.

5 (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the  
10 protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic  
15 residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues,  
20 where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino  
25 acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by  
30 reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

#### **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt,

and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,

41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel

chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

5 In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope  
10 of the invention.

### **Epitopes and Antibodies**

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID  
15 NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses  
20 polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower  
25 stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide  
30 encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies



described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985)). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to

an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very  
5 least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-  
10 2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS),  
15 while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an  
20 immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the  
25 peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example,  
30 polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof,

resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can

also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention); and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from

human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, 5 trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 10 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be 15 specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

20 Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at 25 least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less 30 than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present

invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity

or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent  
5 ligand binding and receptor activation as well as antibodies that recognize the  
receptor-ligand complex, and, preferably, do not specifically recognize the unbound  
receptor or the unbound ligand. Likewise, included in the invention are neutralizing  
antibodies which bind the ligand and prevent binding of the ligand to the receptor, as  
well as antibodies which bind the ligand, thereby preventing receptor activation, but  
10 do not prevent the ligand from binding the receptor. Further included in the invention  
are antibodies which activate the receptor. These antibodies may act as receptor  
agonists, i.e., potentiate or activate either all or a subset of the biological activities of  
the ligand-mediated receptor activation, for example, by inducing dimerization of the  
receptor. The antibodies may be specified as agonists, antagonists or inverse agonists  
15 for biological activities comprising the specific biological activities of the peptides of  
the invention disclosed herein. The above antibody agonists can be made using  
methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.  
5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.  
58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu  
20 et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-  
3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J.  
Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241  
(1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al.,  
Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998);  
25 Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference  
herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited  
to, to purify, detect, and target the polypeptides of the present invention, including  
both in vitro and in vivo diagnostic and therapeutic methods. For example, the  
30 antibodies have use in immunoassays for qualitatively and quantitatively measuring  
levels of the polypeptides of the present invention in biological samples. See, e.g.,



Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and

potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the

hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen-binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies,

including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using  
5 methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and  
10 antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule  
15 in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol.*  
20 *Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule.  
25 Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence  
30 comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be

humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation.

Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected-non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, J. *Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

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*Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that  
5 encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the  
10 nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then  
15 amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized  
20 or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe  
25 specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the  
30 antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for

example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their  
5 entirities ), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the  
10 art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally  
15 occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid  
20 substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other  
25 alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.* 81:851-855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing genes  
30 from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are



derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain  
5 antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the  
10 assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

#### *Methods of Producing Antibodies*

The antibodies of the invention can be produced by any method known in the  
15 art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an  
20 expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using  
25 techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include,  
30 for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or

light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of  
5 the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a  
10 polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

15 A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the  
20 invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems  
25 infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,  
30 BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the

vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993);

Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

5       The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in  
10       culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

      The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second  
15       vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic  
20       free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

      Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any  
25       method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or  
30       fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991);

Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, *supra*, the polypeptides corresponding to a polypeptide,  
5 polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to  
the above antibody portions to increase the *in vivo* half life of the polypeptides or for  
use in immunoassays using methods known in the art. Further, the polypeptides  
corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody  
portions to facilitate purification. One reported example describes chimeric proteins  
10 consisting of the first two domains of the human CD4-polypeptide and various  
domains of the constant regions of the heavy or light chains of mammalian  
immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The  
polypeptides of the present invention fused or conjugated to an antibody having  
disulfide- linked dimeric structures (due to the IgG) may also be more efficient in  
15 -binding and neutralizing other molecules, than the monomeric secreted protein or  
protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In  
many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and  
thus can result in, for example, improved pharmacokinetic properties. (EP A  
232,262). Alternatively, deleting the Fc part after the fusion protein has been  
20 expressed, detected, and purified, would be desired. For example, the Fc portion may  
hinder therapy and diagnosis if the fusion protein is used as an antigen for  
immunizations. In drug discovery, for example, human proteins, such as hIL-5, have  
been fused with Fc portions for the purpose of high-throughput screening assays to  
identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58  
25 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be  
fused to marker sequences, such as a peptide to facilitate purification. In preferred  
embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the  
tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA,  
30 91311), among others, many of which are commercially available. As described in  
Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-  
histidine provides for convenient purification of the fusion protein. Other peptide tags



useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof  
5 conjugated to a diagnostic or therapeutic agent. The antibodies can be used  
diagnostically to, for example, monitor the development or progression of a tumor as  
part of a clinical testing procedure to, e.g., determine the efficacy of a given  
treatment regimen. Detection can be facilitated by coupling the antibody to a  
detectable substance. Examples of detectable substances include various enzymes,  
10 prosthetic groups, fluorescent materials, luminescent materials, bioluminescent  
materials, radioactive materials, positron emitting metals using various positron  
emission tomographies, and nonradioactive paramagnetic metal ions. The detectable  
substance may be coupled or conjugated either directly to the antibody (or fragment  
thereof) or indirectly, through an intermediate (such as, for example, a linker known  
15 in the art) using techniques known in the art. See, for example, U.S. Patent No.  
4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics  
according to the present invention. Examples of suitable enzymes include horseradish  
peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase;  
examples of suitable prosthetic group complexes include streptavidin/biotin and  
20 avidin/biotin; examples of suitable fluorescent materials include umbelliferone,  
fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine  
fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material  
includes luminol; examples of bioluminescent materials include luciferase, luciferin,  
and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$   
25 or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic  
moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or  
a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin  
or cytotoxic agent includes any agent that is detrimental to cells. Examples include  
30 paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin,  
etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin,  
dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-

dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld

et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### *Immunophenotyping*

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to

prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

## 5 *Assays For Antibody Binding*

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent  
10 assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular  
15 Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium  
20 deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or  
25 more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing  
30 the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g.,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

#### *Therapeutic Uses*

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the

antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

### Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or

prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or



indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered  
5 *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct  
10 injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,  
15 e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for  
20 cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.,  
25 Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral  
30 genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can

be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy.

Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be

carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted.

10 The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

25 In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of

the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

5 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

#### *Demonstration of Therapeutic or Prophylactic Activity*

10 The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect  
15 of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient  
20 tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### *Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by  
25 administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses,  
30 chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above;

additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein

and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliet et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term

"pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry

lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.



The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval  
5 by the agency of manufacture, use or sale for human administration.

### *Diagnosis and Imaging*

Labeled antibodies, and derivatives and analogs thereof, which specifically  
10 bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body  
15 fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder,  
20 comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With  
25 respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier  
30 thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art

(e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; 5 radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval 15 following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject 20 has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce 25 diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{mTc}}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor 30 imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging:

The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled-artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### *Kits*

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present

invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

10 In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

15 In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

20 In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled

monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### **Fusion Proteins**

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target

cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur  
5 through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the  
10 polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in  
15 chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be  
20 more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a  
30

fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available.

As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

#### **Vectors, Host Cells, and Protein Production**

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the

vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.



Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A

main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an

expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and  
5 immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example,  
10 techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411,  
15 published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using  
20 techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid  
25 analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine,  
30 norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino

acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used,

depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an  
5 average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000,  
10 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug.*  
15 *Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those  
20 skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated  
25 polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for  
30 therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG-activated-with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention

relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least  
5 dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and  
10 fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing  
15 polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at  
20 least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional  
25 embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as,  
30 for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed



when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al.,

Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described  
5 in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

10 Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by  
15 reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations  
20 proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers  
25 of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues  
30 located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely

modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

### Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers,

since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

5 Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an  
10 amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping  
15 strategies that can be used include in situ-hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

20 Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon  
25 Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

The polynucleotides of the present invention would likewise be useful for  
30 radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London

(1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

5        Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins  
10    University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

      Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.  
15    First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide  
20    and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

      Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using  
25    polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

      Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the  
30    present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression

level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe  
5 containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand  
10 containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present  
15 invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the  
20 polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard  
25 being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

30 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples

include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art.

5 Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a  
10 "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying  
15 disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or  
20 according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain  
25 components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and  
30 tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide

backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

10       The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic  
15       leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often  
20       associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by  
25       insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in  
30       some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)



For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information

disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR

Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be  
5 used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the  
10 present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a  
15 specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

20

### Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a  
25 biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay  
30 (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and

technetium ( $^{99m}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo  
5 imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for  
10 the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99m}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or  
15 intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99m}\text{Tc}$ . The labeled antibody or antibody fragment will then  
20 preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

25 Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is  
30 indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for

detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

5           Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair  
10   proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune  
15   response to proliferative cells or tissues).

          Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can  
20   activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

          At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also  
25   be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

### Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrün et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations,

lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and  
5 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3,  
10 pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters  
15 include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters,  
20 such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the  
25 polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver,  
30 spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular,

fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.



In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA, 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP

starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta*,

394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and  
5 reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ratio will be from about 5:1 to about 1:5. More preferably, the  
10 ratio will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469  
15 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

20 In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis  
25 virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-  
30 19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any

means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and  $\text{CaPO}_4$  precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

5       The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

10       In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional  
15       mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991);  
20       Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA, 76:6606 (1979)).

      Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993);  
25       Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and  
30       constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other

varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

5       The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor  
10    factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein.

15    Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

20       Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle  
25    accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium  
phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the  
30    rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is

administered by direct injection into or locally within the area of arteries.

Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide  
5 construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include  
10 recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection,  
15 aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA, 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present  
20 invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

25 Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of  
30 polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of



the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

## 5 **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological  
10 activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with the following systems.

15

## **Immune Activity**

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing  
20 diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of  
25 these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

30 In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an

immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic

mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of

the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, diagnosed  
5 and/or prognosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing  
spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune  
10 thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura),  
autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that  
15 may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid  
syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing  
polychondritis, rheumatic heart disease, neuritis, uveitis ophthalmia,  
polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary  
20 inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that  
may be treated, prevented, diagnosed and/or prognosed with the compositions of the  
invention include, but are not limited to, scleroderma with anti-collagen antibodies  
25 (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed  
connective tissue disease (often characterized, e.g., by antibodies to extractable  
nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by  
nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell,  
microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often  
30 characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility  
(often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often  
characterized, e.g., by glomerular basement membrane antibodies or immune

complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic  
5 drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized,  
10 e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by  
15 myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

20 In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed  
25 using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred  
30 embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

5 In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention

In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive  
10 agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, prognosing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could  
15 be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, Polynucleotides, polypeptides, antibodies, and/or  
20 agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

25 Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic  
30 molecule, or blood group incompatibility.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, may be used to treat, prevent, diagnose and/or prognose

IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

5           Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells  
10 involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection,  
15 nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain  
20 injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma,  
25 pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

Because inflammation is a fundamental defense mechanism, inflammatory disorders can effect virtually any tissue of the body. Accordingly, polynucleotides,  
30 polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis,

balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chorditis, cochitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

10 In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues.

15 Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

25 In other embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

30 Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected,



and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune

response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meisseriesia meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and

immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell responsiveness to pathogens.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce higher affinity antibodies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to accelerate recovery of immunocompromised individuals.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment,

compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone  
10 marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or  
15 treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen  
25 presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct  
30 an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to  
5 virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or  
10 Common Variable Immunodeficiency.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies,  
15 polynucleotides and/or agonists or antagonists of the present invention are used in the pretreatment of bone marrow samples prior to transplant.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based therapy for genetically inherited disorders resulting in immuno-  
20 incompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmania.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in one or more of the  
30 applications described herein, as they may apply to veterinary medicine.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of

blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and  
5 diseases/disorders associated with pathogens.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus  
10 and multiple sclerosis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and  
15 blocking sepsis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related  
20 idiopathic monoclonal gammopathies, and plasmacytomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated  
25 and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic  
30 syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit complement mediated cell lysis.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery  
10 wall.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult respiratory distress syndrome (ARDS).

In another specific embodiment, polypeptides, antibodies, polynucleotides  
15 and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists  
20 thereof are used to diagnose, prognose, treat, and/or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne  
25 infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media,  
30 conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with polynucleotides or

polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

5 In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

10 In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, acute  
15 myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of  
25 decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete  
30 splenectomy.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of



the present invention (e.g., Fc fusion protein; see, e.g., Example 9). Agonists of the invention include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins; see, e.g., Example 9). polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention  
5 may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic  
10 animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741).  
15 Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention in an organ system listed above.

## 20 **Blood-Related Disorders**

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hemostatic (the stopping of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists  
25 or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or  
30 antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be

important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used for the prevention of occlusion of saphenous grafts, reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis

and/or treatment of anemias and leukopenias described below. Alternatively, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g.,  
5 basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for example eosinophilia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists  
10 of the present invention may be used to prevent, treat, or diagnose blood dyscrasia.

Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The polynucleotides, polypeptides, antibodies,  
15 and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic  
20 acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hemolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may  
25 be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias  
30 arising from drug treatments such as anemias associated with methyl dopa, dapsone, and/or sulfadruugs. Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing,

and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

5 The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating  
10 thalassemias, including, but not limited to major and minor forms of alpha-thalassemia and beta-thalassemia.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited  
15 to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thromboasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophelias such as hemophelia A or Factor  
20 VII deficiency and Christmas disease or Factor IX deficiency, Hereditary Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

The effect of the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention on the clotting time of blood may be monitored  
25 using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in  
30 a specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction